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EARLY INCIDENCE OF SPONTANEOUS MEDIAL DEGENERATION ("ARTERIOSCLEROSIS") IN THE AORTA OF THE RABBIT

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NEW YORK

In the course of an attempt to reproduce the transmission of viosterol-induced arteriosclerosis from mother to young as described by Junck,¹ the opportunity presented itself to study the aortas from 125 young rabbits. These rabbits, with few exceptions, were the successive litters of several mothers by a single father. Coming from known stock and being raised under standard conditions, they formed unusually well controlled material for study. The remarkably high incidence of degenerative changes in the media of the aortas of these rabbits forms the basis for this report.

METHOD

The parent rabbits, 6 does and 1 buck, were large, young adult chinchillas.² Two females received subcutaneously 0.18 cc of 1,500 D viosterol^{2a} (30,000 rat units), divided into daily doses over a period of one week, 2 received 12 cc (200,000 rat units) in similar fashion. The remaining 2 females served as controls, they as well as the male received no viosterol. At periods varying from one month before to eight months after the administration of the viosterol, the animals were bred. The control mothers were bred at the same time, and also again nearly a year after the beginning of the work. The animals were housed in large breeding cages in a sunny indoor environment. The young were weaned at 6 weeks of age and placed on the standard stock diet fed the parents.³ The successful breeding of the adults and the satisfactory growth and excellent nutritional state of the young indicated that the diet was a liberal one. The incidence of coccidiosis was sharply limited by the exclusion of fresh vegetables.

This investigation was aided by a grant from the Josiah Macy Jr Foundation. From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

1 Junck, A. *Virchows Arch f path Anat* **283** 265, 1932.

2 The parent rabbits were of a strain used for breeding purposes at Rockland Farms, New City, N. Y.

2a Viosterol was supplied as 1,500 D vigantol by the Winthrop Chemical Co., New York.

3 The stock diet for rabbits was composed as follows: oats 50 per cent, rolled oats, 10 per cent, whole wheat, 10 per cent, barley, 10 per cent, bran, 5 per cent, corn 5 per cent, linseed oil meal, 5 per cent, and molasses-alfalfa (equal parts), 5 per cent. Timothy and alfalfa hay were given ad libitum.

At ages varying from 1 day to 8 months the rabbits were killed and autopsies performed. In the examination of the younger animals the entire aorta was sectioned serially, in that of the older ones, suitable blocks were removed from the aorta and similarly sectioned.

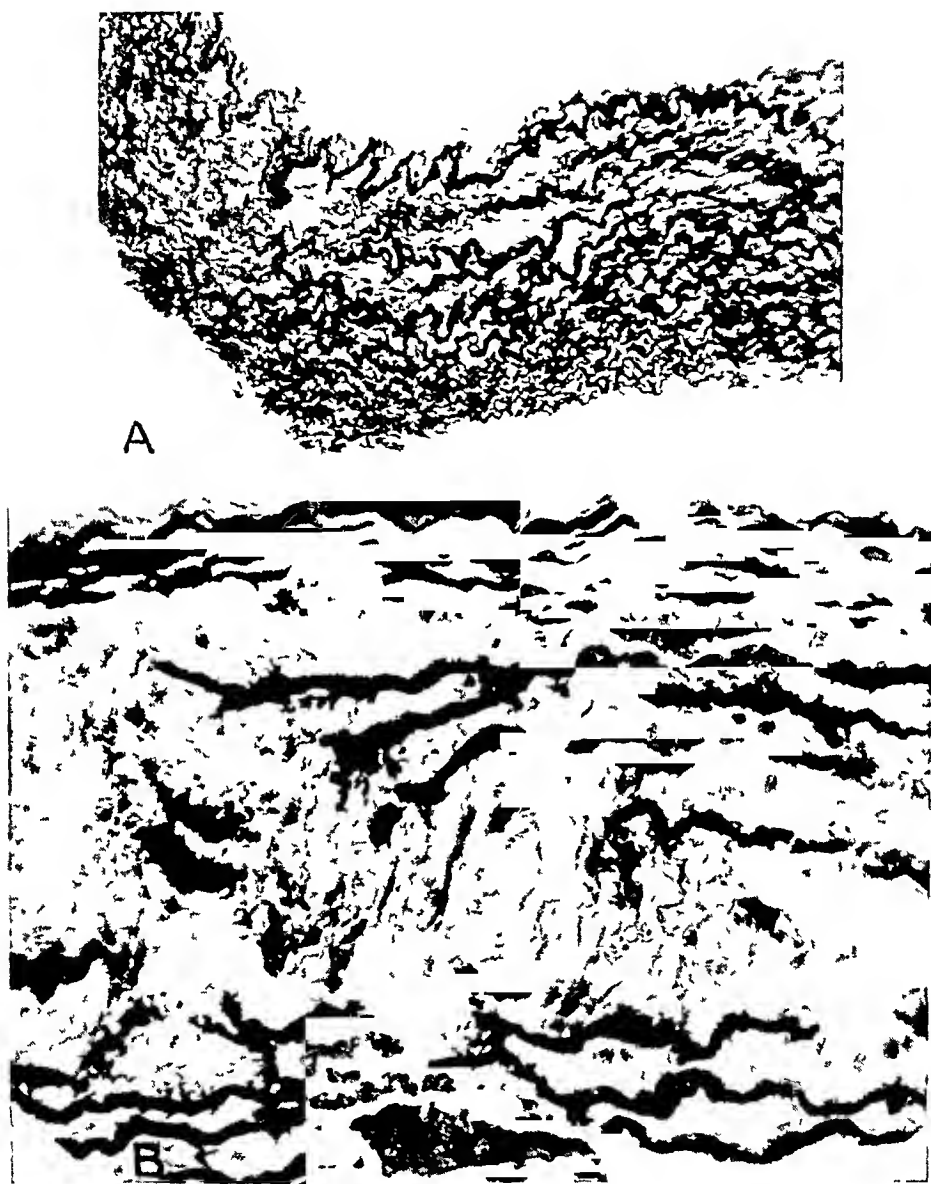


Fig 1—*A*, separation, fragmentation and fraying of the medial elastic fibers in the aorta of a 10 day old rabbit. Weigert-Van Gieson stain, $\times 160$. *B*, lesion in a 23 day old rabbit. Delicate elastic fibrils run transversely between interrupted elastic fibers. Note the mononuclear infiltration. Weigert-Van Gieson stain, $\times 450$.

RESULTS

In gross character the aortic lesions ranged from a single minute, slightly raised, grayish-white, round spot, usually in the ascending portion of the arch, to numerous discrete or confluent, round or linear,

wrinkled, often umbilicated, gray or occasionally yellowish, partly calcified plaques, varying in size up to 2 mm in diameter and involving most of the arch

Histologic study indicated that the earliest change was swelling, fragmentation and fraying of the elastic fibers in the inner media (fig 1 *A*) This was associated with an increase in interfibrillar fluid

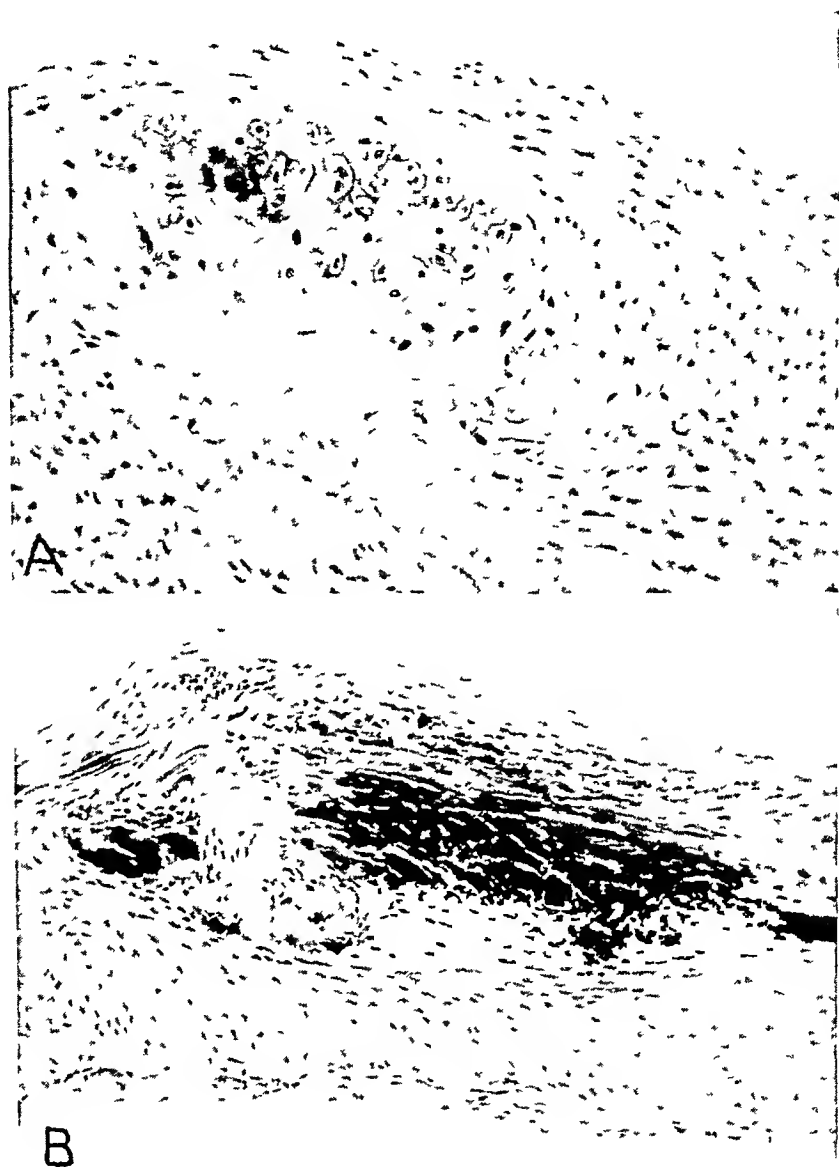


Fig 2—*A*, formation of cartilage in a medial aortic lesion of an 8 month old rabbit Hematoxylin-eosin stain, $\times 160$ *B*, extensive calcification of a lesion at 5 months Hematoxylin-eosin stain, $\times 100$

There ensued an infiltration of wandering mononuclear cells between the altered elastic fibers Often bundles of delicate wavy elastic fibrils ran transversely (radially) between the wandering cells (fig 1 *B*)

Hyalinization of the interfibrillar ground substance, mucinous degeneration and calcification (fig 2 B) occurred at later stages. Calcium was first laid down along and closely apposed to swollen, taut or fragmented elastic fibers. The presence of fat was not a feature, it was seen only occasionally in late lesions within phagocytes or scattered between the fibers. Small groups of cartilage cells were sometimes observed in the older plaques (fig 2 A). The intima over the lesions was usually raised and later wrinkled. There was a moderate tendency for the endothelial

*Occurrence of Aortic Medial Degeneration in Young Rabbits from Mothers
Given Viosterol Prior to First Breeding*

Mother and Amount of Viosterol in Rat Units	Litter	Period Elapsing Between Administration of Viosterol to Mother and Birth of Litter, Months	Results of Postmortem Examination of Aortas of Young at Ages Given in Months							
			$\frac{1}{2}$	1	2	3	4	5	6	8
A 30,000	1	0*								
	2	6		+	+					
	3	9	-	+	-	+	+		+	+
B 30,000	1	0	---							
	2	6	---	+		+	+	+		+
	3	9	---		+	+	+	+		+
C 200,000	1	6	---	---	+	+				
	2	9		---		+	+		+	+
D 200,000	1	2	---	---			+		+	+
	2	6		---		+		+		+
	3	9		---			+			+
X Control	1*		---	---	---	---		+	---	+
	2		---	---	---	+	---	+		+
	3			---	+	+	+	+		+
	4			---	++	---	---	---		---
Y Control	1*		---							
	2		---							
	3		+	---			+			
	4			---		++	+			+
	5			---	+	+	+			+

* Mother received pregnant father of litter unknown

+ Medial degeneration of aorta present

— Medial degeneration of aorta absent

cells to multiply, forming a layer usually one cell thick with nuclei relatively close together. Only rarely was actual thickening of the intima to be observed. Pertinent lesions were not seen in any of the viscera.

The occurrence of aortic lesions in the 125 young rabbits studied is given in the table. The youngest animal in which a lesion was seen was only 10 days old. It was from a mother which did not receive viosterol (fig 1 A). The aorta of another animal, killed at 23 days of age, from another litter of the same mother, also contained a single definite lesion, with a spreading apart and splitting of the elastic lamellae (fig 1 B). Fourteen rabbits were killed at the age of 1 month,

6 of them from mothers which had received viosterol and 8 from the 2 control mothers. Three of the former and 4 of the latter, or one half of the 14, presented lesions. At 8 months of age, practically all the animals in this stock had aortic changes. In general the older the animal the more marked were the changes, although deposits of calcium were observed in the young from both the viosterol treated and control mothers as early as at 3 months of age. The incidence of the lesions at various ages is graphically summarized in figure 3.

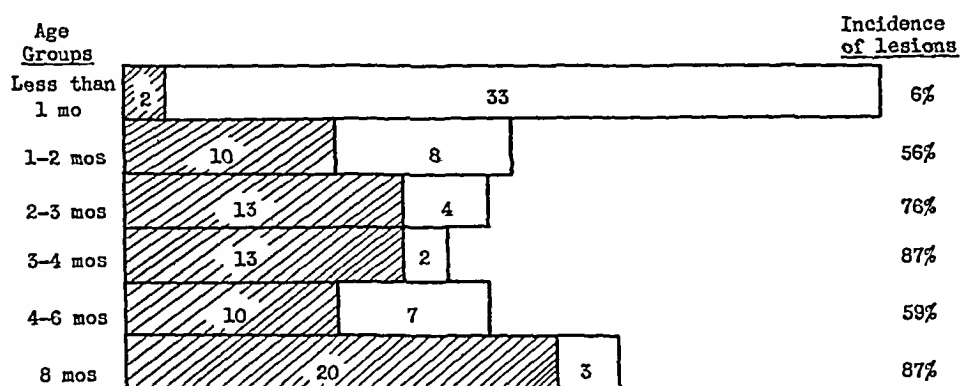


Fig 3—Incidence of aortic medial degeneration among young rabbits, classified according to age. In this and figure 4, the shaded areas represent the number of rabbits presenting lesions, the unshaded, the number with normal aortas.

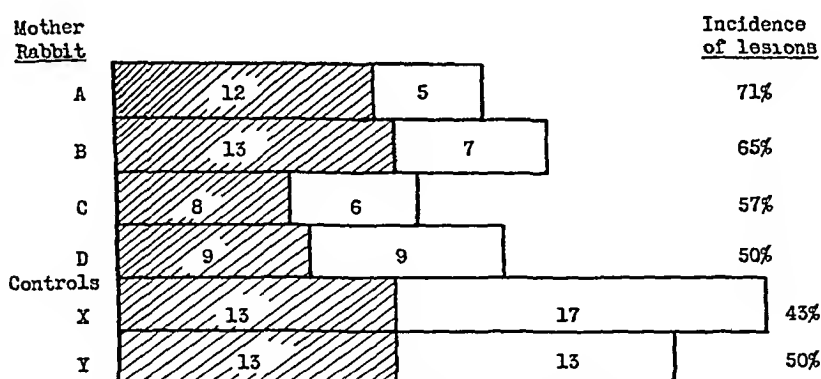


Fig 4—Incidence of aortic medial degeneration among young rabbits, classified according to the mother. Four mothers (A, B, C and D) received viosterol prior to the first breeding.

The incidence of the lesions in the young from the several mothers is summarized in figure 4. It ranges from 71 per cent in the young from mother A to 43 per cent in those from 1 of the control mothers. Reference to the table shows that all but 2 of the young from mother A were killed at ages of 1 month or more, which probably accounts for the high incidence in this family. The variations between the other 5 families are probably without significance. The total incidence in the entire group of 125 rabbits is 54 per cent.

At the end of the experiment, the mothers were killed and autopsies performed. The only significant lesions were in the aortas. Those from the 2 rabbits (A and B) which received 30,000 rat units of viosterol presented the greatest degree of change with numerous partly calcified small plaques in the arch. The lesions in rabbit C, which received 200,000 rat units, were somewhat less marked. No changes were demonstrable, even microscopically, in the aorta from rabbit D (200,000 units). The aortas of the 2 control mothers presented moderate changes, comparable to those in rabbit C. Thus no correlation is possible between the dose of viosterol, the amount of sclerosis in the mother's aorta and that in the aorta of the young. Although it is somewhat difficult to calculate in terms of rat units the doses of viosterol used by Junck¹ and by Schmidtman (quoted by Junck), they seem to fall within the same range or somewhat under that which was used in this investigation.⁴

Junck gave a "total of 0.5 mg of irradiated ergosterol during eight days, as standardized vigantol," probably a total dose of about 10,000 rat units. He gave also to another rabbit "2 drops of standardized vigantol."

The present attempt, then, to reproduce the transmission of sclerosis due to viosterol from mother to young must be deemed unsuccessful. All the lesions in the aforementioned animals are to be considered as spontaneous.

COMMENT

In the fifty-three years which have elapsed since Israel's⁵ original description of medial degeneration and calcification in the rabbit's aorta, under the name of chronic endoaortitis deformans, similar changes have been reported following a great variety of experimental procedures, including the use of bacterial, chemical and physical stimuli. Judging from the descriptions and illustrations, many of the lesions were entirely comparable to those now recognized to occur spontaneously in the rabbit's aorta. There was a wide quantitative variation in this recognition, however, ranging from none to 45.5 per cent of the rabbits. The literature has been largely summarized by Newburgh and Clarkson,⁶ Dominguez,⁷ Nuzum and his co-workers⁸ and Benson and his co-workers.⁹ The majority of observers saw spontaneous medial

4 Schmidtman, as quoted by Junck,¹ gave "4 mg of irradiated ergosterol daily for twelve days in the form of 10 drops of 1 per cent vigantol." According to the Winthrop Chemical Co., 1 per cent vigantol in 1929 was probably about 1,500 D (180,000 rat units per cubic centimeter).

5 Israel, O. *Virchows Arch f path Anat* **86** 299, 1881.

6 Newburgh, L. H., and Clarkson, S. *Arch Int Med* **31** 653, 1923.

7 Dominguez, R. *Arch Path* **5** 577, 1928.

8 Nuzum, F. R., Elliot, A. H., Evans, R. D. and Priest, B. V. *Arch Path* **10** 697, 1930.

9 Benson, R. L., Smith, K. G., and Semenov, H. *Arch Path* **12** 924, 1931.

changes in less than 10 per cent of normal control rabbits. Dominguez found the mean for more than 3,500 rabbits examined during the preceding twenty years in this country and in Europe to be less than 6 per cent.

The earliest report of the recognition of aortic medial degeneration in normal rabbits appears to be that of Fischer,¹⁰ in 1905, who established that lesions of the media of the aorta similar to those resulting from epinephrine may occur spontaneously. However, Kaiserling,¹¹ in 1907, in the course of a cautious evaluation of the then current enthusiasm for sclerosis from epinephrine stated that several years previously he had seen a calcified aorta in a stock rabbit. In 1907 Miles¹² drew down on herself a fire of comment by reporting from Colorado the occurrence of medial aortic lesions in 17 of 49 normal rabbits, that is, in 34.8 per cent. Miller,¹³ in a letter in the same year, objected to Miles' conclusions, emphasized the failure of previous workers, including Jores,¹⁴ to observe spontaneous lesions and expressed his belief that Miles' animals were unusual and probably associated with an abnormal environment. In the summarizing table published by Dominguez, Miles' figure of 34.8 per cent was conspicuous as the highest, with the next 15.4 per cent that from unpublished data by Goldblatt. Levin and Larkin,¹⁵ 1910, were quoted as observing that 12.9 per cent of normal rabbits have spontaneous aortic lesions. But a perusal of the latter's paper reveals that of 240 rabbits "used for laboratory purposes mainly for obtaining blood serum, and with no toxic substances of any kind introduced" 31, or 13 per cent, had gross aortic lesions and were not examined further. The aortic arch of the remaining 209 animals was examined microscopically. Seventy-eight of the 209 rabbits, or 32.5 per cent of the original 240, presented microscopic lesions, making a total incidence of 45.5 per cent. Inasmuch as the exact handling of these animals was not specified, one cannot be certain that all of these lesions were spontaneous, but the significant point remains that many of them were noted only after microscopic study. Microscopic studies of the aorta in the several other normal series in the literature appear to have been limited to details of lesions noted grossly.

Of the 68 involved aortas in the series of 125 rabbits of this study, 10 had a normal gross appearance, the medial lesions being noted only in microscopic sections. In 7 other aortas the gross conditions were recorded as questionable and a definite diagnosis was possible only after microscopic examination.

10 Fischer, B. *Verhandl. d. XXII Kongr. f. inn. Med.* **20** 235, 1905.

11 Kaiserling, C. *Berl. klin. Wchnschr.* **44** 29, 1907.

12 Miles, A. B. *J. A. M. A.* **49** 1173, 1907.

13 Miller, J. L. *J. A. M. A.* **49** 1789, 1907.

14 Jores, L. *Beitr. z. path. Anat. u. z. allg. Path.* **41** 167, 1907.

15 Levin, I., and Larkin, J. *Proc. Soc. Exper. Biol. Med.* **7** 109, 1910.

The explanation of the high incidence of medial degeneration in the aortas of this series of rabbits and of the great differences in incidence reported from various laboratories is not obvious. Faulty diet does not seem to be a factor, as the diet used in this experiment was considered excellent. General living conditions were also ideal, except for relative lack of exercise and of direct sunshine. There is no apparent seasonal factor. Infection in the ordinary sense, including coccidiosis, can be excluded. A difference in the rabbit strain seems probable in view of the wide geographic differences in incidence. The observation of Zeek¹⁶ that inbreeding may produce a strain relatively free from spontaneous sclerosis is very illuminating. The lesion pathologically appears to be a toxic degeneration. If this is true, it furnishes another example of the relationship of heredity and susceptibility to disease.

SUMMARY

A study was made of the aortas of 125 apparently normal young rabbits aged from 1 day to 8 months and of known parentage. They represented the successive litters of 4 female rabbits given viosterol prior to the first breeding and of 2 untreated female rabbits. Spontaneous medial degeneration, or "arteriosclerosis," was observed in 68 of the 125 rabbits or in 54 per cent. No significant difference was demonstrable between the young from the treated and the untreated mothers. Lesions were found at ages as early as 10 days. The incidence increased with age to 87 per cent at 8 months. Microscopic examination was necessary for a positive diagnosis in 25 per cent of the lesions.

16 Zeek, P. Arch. Path. 16 302, 1933

POSSIBLE FUNCTIONAL SIGNIFICANCE OF THE LONGITUDINAL MUSCLE IN THE ADRENAL VEINS IN MAN

ISOLDE T ZECKWER, M D

PHILADELPHIA

The peculiar structure and arrangement of the longitudinal muscle bundles in the veins within the adrenal medulla in man must indicate a function of considerable importance. The observers who have studied these structures histologically have proposed theories which do not seem adequate. Since the ordinary laboratory animals show only slight development of these muscle bundles, the experimental attack on the problem of their function is impossible.

Recently Bauer, Dale, Poulsson and Richards¹ studied with great care and ingeniousness the control of the circulation through the liver in the dog and clearly demonstrated a mechanism determining the rapid outflow of blood which depends on the peculiar structure of the longitudinal muscle bundles in the hepatic veins of the dog. Since there is considerable similarity between these muscles in the hepatic veins of the dog and those in the adrenal veins in man, it seems altogether possible that the precise experimental results obtained by Bauer and his co-workers in explaining the functional significance of the hepatic veins in the dog can be applied to the consideration of the function of the adrenal veins in man. Without the possibility of direct experimental attack, the formation of a hypothesis depending on an analogy in structure seems justifiable.

In many adrenals removed from the human adult the following features may be recognized in sections prepared in a routine way from fixed tissues. The veins within the medulla in cross-section show substantial bundles of muscles running longitudinally along the length of the vein (fig 1 *A*). These bundles occur at irregular points, and between the muscle bundles the wall of the vein is thin and lacking in muscle. The longitudinal bundles protrude markedly into the lumen and nearly touch the endothelium on the opposite side of the lumen. No circular muscle fibers are presented. Passing into these muscular veins are smaller blood vessels with very thin walls or merely with endothelium (fig 1 *B*). As these vessels enter a larger vein, they pass

From the Department of Pathology, School of Medicine, University of Pennsylvania

1 Bauer, W , Dale, H H , Poulsson, L T , and Richards, D W J Physiol
74 343, 1932

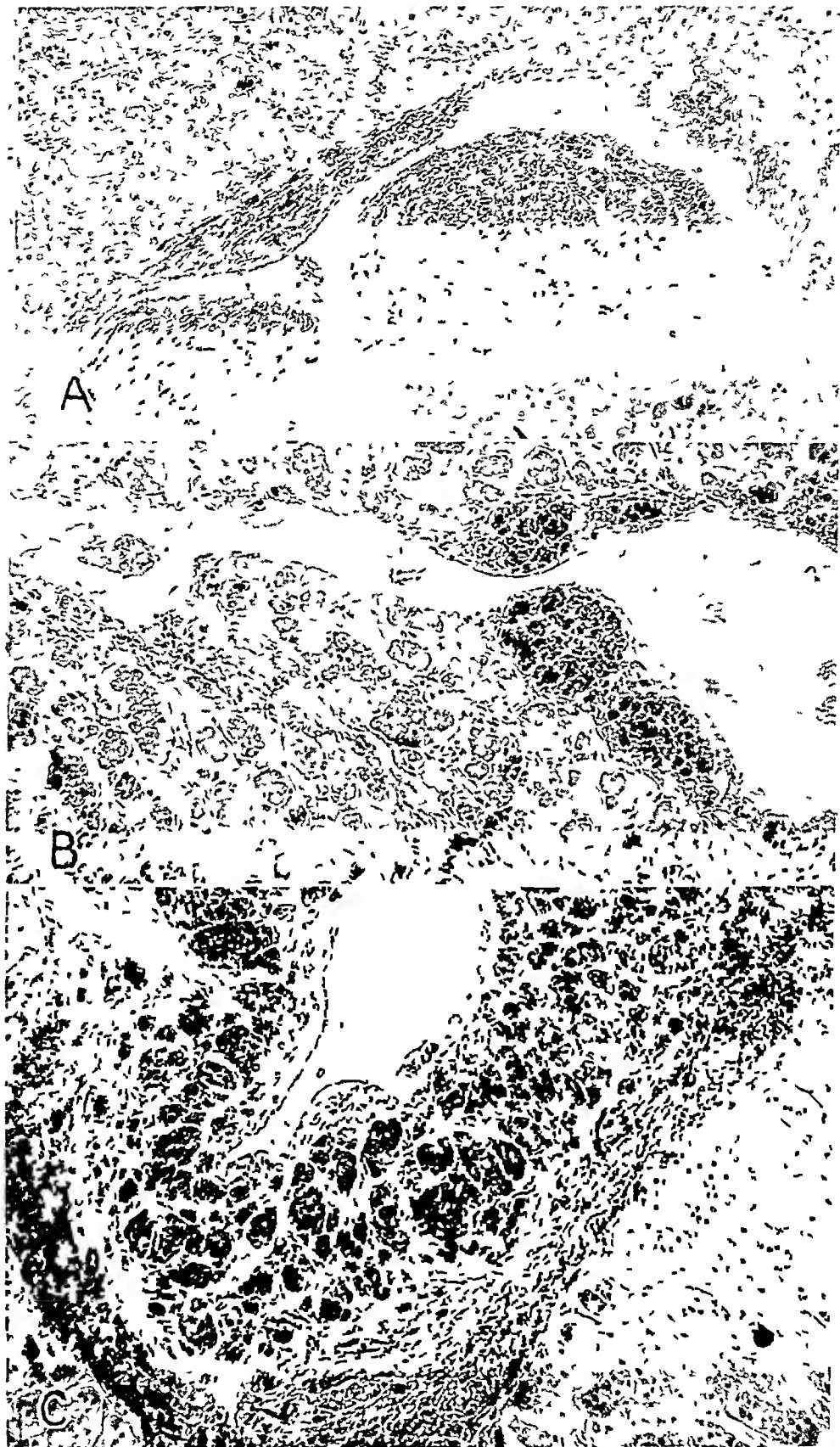


Fig 1—*A* shows longitudinal muscle seen in cross-section protruding into the lumen of a vein in the adrenal gland of a human being, *B*, the entrance of tributaries into human adrenal vein of medium size guarded by muscle bundles, and *C*, the main adrenal vein with substantial bundles of longitudinal muscle completely surrounding the lumen

between muscle bundles, so that the orifices are actually encroached on by the muscles in the larger vessels, and the obvious dilatation of the small vessels proximal to their entrance into the larger vein gives clear evidence that the muscle is actually causing functional obstruction to the tributaries. As the main adrenal vein is approached, the muscle bundles increase in size and surround the lumen almost symmetrically (fig 1 C). The larger hepatic veins of the dog (fig 2 A) are similar in structure to the main adrenal vein. The peculiar asymmetrical arrangement of muscle in the smaller tributaries of the adrenal vein in man is a development far beyond anything seen in the hepatic veins of the dog. According to Popper,² however, if the dog's liver is fixed at a time when the muscle in the hepatic veins is contracted projections into the lumen are seen, so that in cross-section the lumen appears star-shaped. This effect is shown in figure 2 B. In figure 2 C are seen longitudinal muscles extending the length of two hepatic veins which are tributaries to the vena cava of the dog.

Before considering the application of the results obtained by Bauer to the adrenal in man, a summary is presented of the previous suggestions as to the significance of the musculature of the adrenal veins. Ferguson³ stated that "the peculiar longitudinal arrangement of the muscular tissue, the valve-like protuberances at the junctions of the venous vessels, the absence of circular muscle from the walls of the veins of all sizes, and the general appearance of these vessels which are so remarkably different from the veins of most other organs, become, to say the least, extremely significant of a close structural relation, physiologically speaking, to the presence of an astringent secretion in the outflowing blood current." He offered, however, no explanation of how these mechanical features are effective.

Poll⁴ expressed the belief that the musculature is a means of securing a smooth and equal outflow of blood. Peindarie⁵ maintained that the muscles of the medium-sized branches act as valves obstructing the flow from the smaller to the larger veins and that the powerful musculature expresses the contents of the larger veins into the general circulation. Maresch,⁶ from serial sections and wax reconstructions, concluded that when the longitudinal muscles contract the thickening and shortening of the fibers result in narrowing or complete closure of the lumen. At the points at which the smaller tributaries pierce the muscle bundles in reaching the lumen of the larger vessel, contrac-

2 Popper, H. *Klin Wchnschr* **10** 2129, 1931

3 Ferguson, J. S. *Am J Anat* **5** 63, 1906

4 Poll, H. *Berl klin Wchnschr* **46** 1973, 1909

5 Peindarie, J. *Compt rend Soc de biol* **83** 958, 1920

6 Maresch, R. *Wien klin Wchnschr* **34** 44, 1921

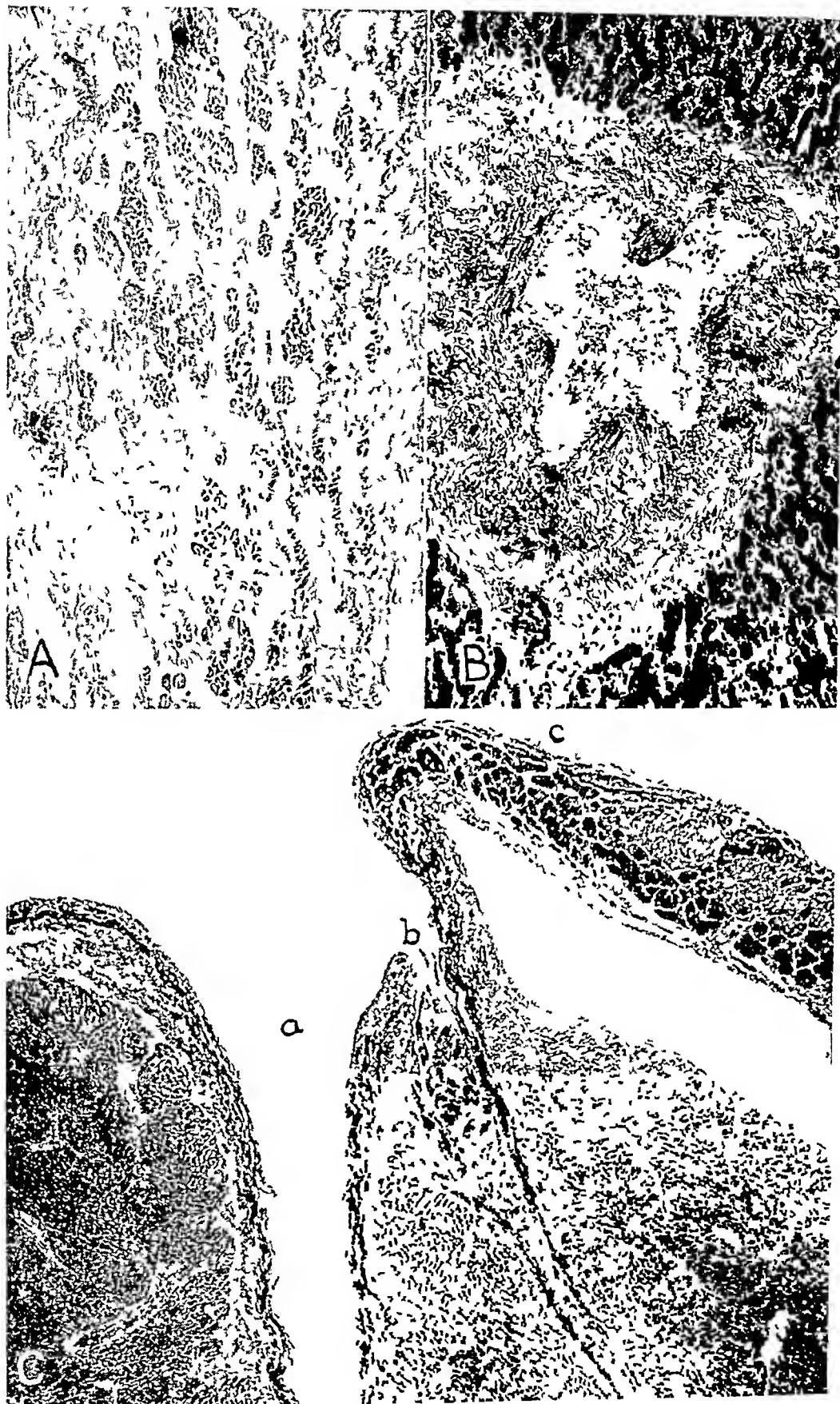


Fig 2—*A* shows bundles of longitudinal muscle seen in cross-section in a large hepatic vein of a dog, *B*, a cross-section of a small hepatic venous tributary in a dog with a stellate lumen and bundles of longitudinal muscle in the four pillars which project into the lumen, and *C*, longitudinal muscles seen in longitudinal section in two hepatic veins (*a*) and (*b*). The muscles are continuous with the longitudinal muscle seen in cross-section in the vena cava (*c*). Mallory's differential connective tissue stain

tion of the muscle holds back the contents of the smaller vessel, which is seen to undergo sinuous widening. Under stimulation of the sympathetic nerve supply, contraction of the muscles hinders outflow of the blood and thereby enriches its epinephrine content which is available for subsequent discharge. Maresch, however, suggested another explanation, namely, that by contraction of the musculature the organ is squeezed as a sponge in the direction of the central vein. Against this theory is the fact that the muscle bundles become larger as the main vein is approached, so that shortening and thickening of the muscles during contraction should cause progressively more complete occlusion of the lumen as the hilus is approached.

Kutschera-Aichbergen⁷ accepted Maresch's view that the muscles in the central vein block the tributaries and cause damming of the blood in the medulla. Maresch had suggested that the blood held back by the contracted veins might find its way into anastomoses outside the adrenal gland. Kutschera-Aichbergen enlarged on this idea, and, by injection of a dye into the left adrenal vein, he observed that the dye passed through anastomoses of the capsular vessels into the pancreas and liver. He therefore expressed the belief that with contraction of the muscle in the adrenal veins the current of blood is reversed and epinephrine is forced through the anastomoses into the portal circulation. He stated that epinephrine injected into the portal circulation is more effective in producing hyperglycemia than when introduced into other veins, and, moreover, that epinephrine introduced into the portal vein does not raise the blood pressure as it does when introduced into other veins. He assumed that the muscles in the adrenal veins contract under stimulation of the sympathetic nerves.

Henderson,⁸ from the study of wax reconstructions, stated that when two veins approach the point of anastomosis the longitudinal fibers in each vessel "fuse in the angle of anastomosis." He expressed the belief that the "significance of this peculiar muscular arrangement in the wall of the central veins of the suprarenal gland probably consists in the fact that contraction of the muscles results in active dilatation of these veins." His conception is best understood by consulting his diagram. He suggested that "this mechanism regulates the outflow of blood" under sympathetic nervous stimulation. This explanation can apply only to cases in which each vessel before joining the other contains muscle; it does not explain what happens as the result of frequent entrance of vessels containing no muscle into larger veins with muscle surrounding the entrance of the smaller vessel.

7 Kutschera-Aichbergen, H. *Frankfurt Ztschr. f. Path.* **28** 262, 1922.

8 Henderson, E. F. *Anat. Rec.* **36** 69, 1927.

Goldzieher and Sherman⁹ measured the thickness of muscles in material obtained in fifty-one autopsies selected at random but limited to those performed on adults, and he calculated the ratio of the thickness of the muscle to the outer diameter of the vessel. The ratios were greater in cases of hypertension and in those of "renovascular alterations without manifestations of hypertension." They stated "It seems reasonable to assume that such hypertrophy is the result of constant overexertion resulting from the attempt to regulate and hold back the excessive liberation of the pressor glandular discharge." The investigators claimed that their results added "new and weighty evidence to the theory which links hypertension and allied diseases with functional disturbances of the suprarenal glands." In their measurements of muscle there seems to be an error in the unit used for the statement of absolute values. The values of from 31 to 61 microns for the thickness of hypertrophied muscle are obviously too low. However, their relative grouping of musculature as normal and hypertrophied holds in spite of this.

Allen¹⁰ measured by means of a planimeter the muscles and lumens of the veins in eighteen patients with hypertension (average age, 54) and in twenty-five persons in whom the blood pressure was normal (average age, 48). He did not state the absolute measurements, but the ratio of muscle area to the area of the lumen was twice as great in the cases of hypertension. He expressed the belief that the muscular hypertrophy "in all probability indicates increased functional activity, which occurs as a result of sympathetic overactivity."

Allen and Page,¹² assuming that the inferior vena cava has the same intravenous pressure as that of the adrenal veins, measured the thickness of the muscle in the inferior vena cava and observed that it was not increased in patients dying with increased venous pressure. They concluded that the muscular hypertrophy of the adrenal veins in patients with hypertension was not caused by increased intravenous pressure.

Kashiwagi¹³ observed that the muscles of the adrenal veins were hypertrophied in diseases in which he stated that there is an increased epinephrine content of the blood, such as contracted kidney, and that they were less hypertrophied in conditions in which he claimed that there is a decrease in the epinephrine content, but proof is lacking that there are such changes in the epinephrine content of the blood in the conditions he mentioned. Kutschera-Aichbergen⁷ suggested that blocking of the outflow of blood by the musculature of the veins with

9 Goldzieher, M. A., and Sherman, I. *Arch Path* **5** 1, 1928

10 Allen, E. V. *Ann Int Med* **3** 153, 1929

11 Deleted

12 Allen, E. V., and Page, I. H. *Deutsches Arch f klin Med* **168** 193, 1930

13 Kashiwagi, S. *Tr Jap Path Soc* **12** 154, 1922

subsequent engorgement of the capillaries may be responsible for certain cases of adrenal hemorrhage Kraus,¹⁴ in discussing adrenal hemorrhage in adults, stated that certain cases may be due to trauma inducing sudden spastic contraction of the muscles of the veins, with rupture of the vessels that have been overdilated by this blockage

Many of these theories of the function of the venous musculature assume that the muscles of the veins contract under sympathetic stimulation and that contraction aids the outflow of epinephrine There are a variety of proofs of the outpouring of epinephrine, which is known to occur rapidly under sympathetic stimulation in various emergencies But it is difficult to visualize the mechanism by which contraction of bulky muscles can increase venous outflow In fact, there is every indication from microscopic sections that contraction by thickening the muscles occludes the lumen and hinders outflow From sections it is clear that at the point at which a tributary enters a larger vein between muscle bundles it is obstructed and dilates proximally and that this thin-walled dilatation is structurally suited to act as a reservoir (fig 1 *B*) Allen¹⁰ observed that the total area of the lumens of all veins increased in the adrenals in which the muscle of certain veins showed hypertrophy From microscopic sections it can also be seen that the longitudinal muscle bundles appear to occlude the lumen more completely than would be possible if there were merely a circular arrangement of muscle fibers Even with the maximum constriction of a structure controlled by circular fibers, there still remains a lumen, but when longitudinal muscle bundles placed only at certain points of the wall of the vein thicken by contraction the pliability of the delicate wall at all other points makes it possible for the endothelium on the opposite side of the lumen to fit closely over the protruding muscle The surrounding tissue apparently offers more resistance than the blood in the lumen, as in all the sections the muscle bulges into the lumen rather than indents the surrounding tissue The theory suggested in this investigation for the functional significance of the muscles in the adrenal veins seems to be implied in the work of Popper,² who described what he called the throttle mechanism of the hepatic veins in the dog He was led to this study by the recognition of the similarity in structure to that of the adrenal veins

When muscle hypertrophies one has a right to believe that it does so in response to the demand for increased functional activity But in what way is contraction of hypertrophied muscle effective in this case? Is it possible that these muscles are in a state of more or less

14 Kraus, A Frankfurt Ztschr f Path **43** 372, 1932

constant partial contraction or tonus under normal conditions, which hinders the outflow of blood and keeps the epinephrine content of the blood high within the adrenal as a reservoir, and that sympathetic stimulation under conditions of emergency relaxes the contracted muscles and permits the rapid passive release of blood containing a high concentration of epinephrine from the adrenal gland? Although such an idea seems radical at first glance and diametrically opposed to most of the aforementioned theories, this is exactly what Bauer and his co-workers¹ demonstrated experimentally in the outflow of blood from the hepatic veins in the dog. This phenomenon depends on a musculature in the vein similar to that of the adrenal vein in man. They showed in the isolated perfused liver, in which records were made of the pressure of the blood in the hepatic artery and portal vein and of the volume of the liver, that either small doses of epinephrine comparable to those liberated physiologically or stimulation of the splanchnic nerve greatly increased the outflow of blood from the hepatic veins through the vena cava, in spite of lessened inflow. This action is determined by a mechanism like a sluice located near the caval orifice of the main hepatic veins, which consists of longitudinal muscle bundles in the walls of the veins. Normally the tonus hinders the outflow of blood from the liver. With histamine the muscles contract, with epinephrine or with sympathetic stimulation, they relax, and therefore the outflow is increased. The mechanism permits the accumulation of a reserve in the liver and a release into the general circulation under special conditions. Bauer's paper should be consulted for details of the mechanism and for a review of the previous work bearing on this subject.

Grab, Janssen and Rein,¹⁵ at about the same time, arrived independently at conclusions nearly similar to those of Bauer and his co-workers, and their experiments have the advantage of having been done under more natural conditions. Furthermore, they showed that atropine increased the venous outflow. It seems probable that the effect of atropine indicates that the muscle is normally in a state of partial contraction under parasympathetic stimulation and that relaxation is produced by such a parasympathetic depressant as atropine.

It seems justifiable to attempt an application of these experimental observations on the circulation in the liver of the dog to that in the adrenal in man, since a direct attack on the problem is impossible in the case of the adrenal veins. In many cases in which sympathetic stimuli are known to be effective, they may be opposed by parasympathetic stimuli.

¹⁵ Grab W, Janssen, S, and Rein, H. *Ztschr f Biol* **89** 324, 1929, *Klin Wchnschr* **8** 1539, 1925

One may therefore theorize about the possible effect of parasympathetic innervation of the adrenal, in which the powerful effects of sympathetic stimulation are known. Parasympathetic fibers are known to innervate the adrenals, but their action is not understood. It is conceivable that parasympathetic impulses are responsible for keeping the muscles of the adrenal veins, when these are well developed, under a certain amount of contraction under ordinary circumstances. In consequence, the secreted epinephrine accumulates in the smaller veins and capillaries of the medulla in high concentration. When a sudden emergency arises, sympathetic stimuli relax the muscles of the veins (just as bronchial muscles relax under sympathetic stimulation), and there is a sudden release of blood of high epinephrine content. The suddenness of the release of large quantities of epinephrine which is known to occur under sympathetic stimulation is, in all likelihood, a vasomotor release of secretion held in reserve rather than entirely the effect of the nervous stimulation of cells to hypersecretion, as is often considered the case.

As long ago as 1897, Biedl¹⁶ maintained that the increased outflow of blood from the adrenal on sympathetic stimulation was due to active dilatation of the "adrenal vessels" by fibers in the splanchnic nerve producing dilatation. Burton-Oritz and Edwards¹⁷ confirmed Biedl's statement and remarked "It also seems singular that the excitation of the splanchnic nerve should dilate the blood vessels of the suprarenal gland and constrict those of the other organs innervated by this nerve." Rigler and Rothberger¹⁸ stated that the blood vessels of the adrenal do not contract as a result of the stimuli that ordinarily contract vessels, such as splanchnic stimuli, while substances which ordinarily produce dilatation of blood vessels also dilate the adrenal vessels. In all these pharmacologic experiments the authors do not specify the type of "blood vessels." Yet is not absence of constriction in the veins necessary in order that arterial dilatation may effect a greater outflow?

Feldberg, Minz, and Tsudzimura¹⁹ recently described liberation of acetylcholine from the adrenals of the cat during stimulation of the splanchnic nerve. In this investigation they were not concerned with a vascular mechanism within the adrenals controlling the discharge of the cellular secretion. But from the point of view of the present study,

16 Biedl, A. *Arch f d ges Physiol* **67** 443, 1897

17 Burton-Oritz, R., and Edwards, D. J. *Am J Physiol* **43** 408, 1917

18 Rigler, R., and Rothberger, C. J., in Bethe, A., et al. *Handbuch der normalen und pathologischen Physiologie*, Berlin, Julius Springer, 1927, vol 7, pt 2, p 1028

19 Feldberg, W. Minz, B., and Tsudzimura, H. *J Physiol* **81** 286, 1934

their data on the liberation by the adrenal of acetylcholine is of considerable interest in view of the known relation of acetylcholine to parasympathetic activity

If the studies on the adrenals of animals apply to those of man, the manner in which epinephrine is discharged under sympathetic stimulation becomes clear, but the added factor of venous obstruction in man must be considered. In man, with bulky muscles in the adrenal veins, a check on the rapid discharge of epinephrine occurs which does not exist in animals

There remains to be explained the significance of the hypertrophy of the muscles in the adrenal veins which has consistently been noted in patients with hypertension. There is no clinical or experimental evidence that patients with hypertension have hyperepinephrinemia. To consider that hypertrophy of the adrenal musculature indicates increased discharge of the secretion of epinephrine in patients with hypertension is therefore illogical. Furthermore, on this supposition, if hypertensive patients did have hyperepinephrinemia they would have changes in carbohydrate metabolism, which is not true. May not the hypertrophied muscle of the adrenal veins in a patient with hypertension be a protective phenomenon inhibiting the outflow of epinephrine during episodes of sympathetic stimulation in a person whose blood pressure is already dangerously high? Such a delicate balance between opposing sympathetic and parasympathetic stimuli is known to be effective in averting crises in other organs, graphic illustrations of which have been given by Cannon²⁰. He stated that "when a factor is known which can shift a homeostatic state in one direction it is reasonable to look for automatic control of that factor or for a factor or factors having an opposite effect". In other words, it is now suggested that the hypertrophy of the musculature in the adrenal is a protective phenomenon, occurring as a result of hypertension rather than being causative, a compensating contraction opposing sympathetic activity. In this connection, it was interesting to find a case of extensive development of the muscle of the adrenal veins in a young adult dying of exophthalmic goiter, in which it is known that there is intense activity of the sympathetic nervous system.

Animals are usually free from hypertension and also show an almost negligible development of muscle bundles in the adrenal veins. May it not be possible that in the absence of hypertension there is no compensatory hypertrophy of muscle in the medium-sized veins limiting the outflow of excessive secretion of epinephrine in animals? It is known that in the adrenal gland of the infant the main adrenal vein contains muscle but that the medium-sized veins within the medulla

20 Cannon, W. B. *Physiol. Rev.* 9: 399, 1929

are almost lacking in muscle. This again fits in with the theory that the development of conspicuous muscle bundles is a gradual compensating hypertrophy.

Apparently no quantitative studies have been made of the age at which this hypertrophy takes place.

PRESENT INVESTIGATION

An attempt has been made to study the development of these muscles with age. Adrenal glands obtained in autopsies performed as a routine in the department of pathology were studied at random, with the exclusion of those in which the medulla showed postmortem softening and lesions such as tuberculosis or tumors. One adrenal was studied in each case. In most cases the tissue fixed in Zenker's solution was stained with Mallory's connective tissue stain in order to differentiate the muscle from the connective tissue with certainty. Approximate measurements were made of the area of the muscle in cross-sections of veins. As most of the bundles were in the form of ellipses, occasionally of circles, the long (a) and short (b) diameters were measured by means of an ocular micrometer and the area calculated as $\frac{a}{2} \times \frac{b}{2} \times \Pi$. The values for all the areas of muscle in a single vein were added. Selection was made of the largest venous branch lying within the medulla which showed asymmetrical arrangement of muscle (in order to exclude the main vein, which is symmetrically surrounded by muscle), and vessels with the area of the lumen larger than 0.35 sq mm were excluded. The veins which happened to be seen in longitudinal section were also excluded. The measurements were, of course, merely approximations, but at least they gave a more objective means of comparison than does a classification on the basis of 2 plus, 3 plus, etc. Without serial sections, the largest vein probably was often missed. Consequently, the recording of the absence of musculature or of muscles of small area is of no significance. In cases in which the muscles were well developed, the bundles were obvious in any section. Attention was concentrated on determining at what age muscular development could be noted. Therefore, the observation of well developed muscle at 20 years of age was of more significance than that of little muscle at 50. A chart was constructed (fig 3) which in no sense is intended as an exact quantitative record. It shows, however, that, within the limitations of accuracy mentioned, it is only in the later age periods that well developed muscles can frequently be found. Certain low values observed in those periods may merely indicate that in the single section studied little muscle was noted rather than that the adrenal veins of that person contained no muscle. The range in, not the absolute, values at the differ-

ent age periods is of concern. In the material obtained in one hundred and seven autopsies selected at random, it was observed that there was a preponderance of persons over 35. Thereupon, supplementary material was selected from the adrenals of persons under 35 and included in the general series. Sections from these tissues

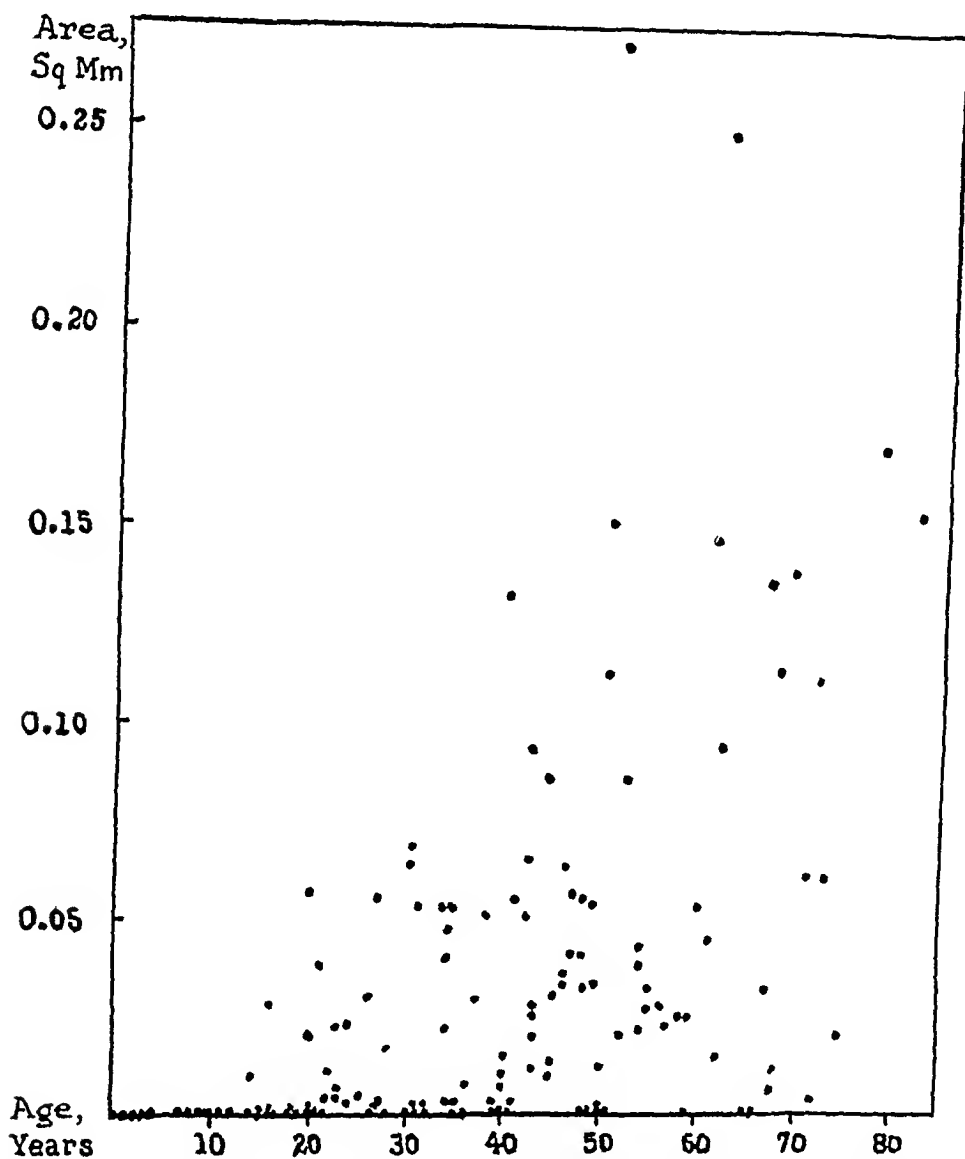


Fig 3—Areas of cross-sections of muscle in veins of medium size (excluding the veins with the area of the lumen exceeding 0.35 sq mm). The single dot in the space representing the 1 year period indicates the average value for the areas obtained in twenty-six children, ranging from new-born infants to those of 11 months. Other single dots in the early age periods usually represent the values obtained for more than one child. After the 12 year period each dot represents the value determined for a single person.

were stained with hematoxylin and eosin instead of with Mallory's connective tissue stain. The total number of cases studied was 172.

SUMMARY

A theory is proposed to explain the rôle which the longitudinal muscles in the adrenal vein in man play in controlling the discharge of epinephrine. It is suggested that under sympathetic stimulation these muscles relax and widen the lumen and that under parasympathetic stimulation they contract and, by their consequent thickening, occlude the lumen.

In the material obtained in a series of autopsies, it was observed that these muscles developed with age.

It is suggested that the hypertrophy of these muscles may be a protective phenomenon, checking the discharge of epinephrine in hypertensive patients.

HORMONAL ORIGIN OF ENDOMETRIOMA

A HYPOTHESIS

J THORNWELL WITHERSPOON, M D

NEW ORLEANS

Endometriosis is a term which was introduced by Sampson to include a variety of adenomatous lesions of the female pelvis the histologic and functional characteristics of which are identical with those of the endometrium. Until 1896 endometriosis was considered to have its origin in the mullerian duct, but in that year, owing to the influence of von Recklinghausen, the wolffian system was considered to be the original source. Then came the intervention of Cullen who firmly established the theory of the origin of adenomyoma from mature endometrium. To Russell belongs the credit of being the first to report the presence of aberrant endometrium in the ovary, a condition originating, in his opinion, from inclusion of the mullerian duct. Since the appearance of Sampson's studies in 1921 this subject has been favored with renewed interest, and at present no gynecological study receives more lively discussion than that of endometriosis.

In Sampson's opinion endometrioma is second only to uterine myoma in frequency. In 119 cases he found the sites of the endometriomas to be as follows: ovaries, 110, uterus, 64, rectovaginal septum, 6, peritoneum, 2, and the abdominal wall in a laparotomy scar, 1. In addition to these locations endometriomas have been found in or on the majority of the pelvic organs.

The etiology of endometrioma is still a matter of debate. Up to the present credit must be given (1) to Cullen¹ for his theory of the mucosal or diverticular origin of diffuse uterine growths, (2) to Ivanoff² for his serosal theory, which draws attention to the proliferative potentialities of the peritoneal mesothelium, (3) to Sampson³ for the hypothesis of tubal regurgitation, or the theory of retrograde menstruation with the direct implantation of living endometrial grafts, and (4) to Robert Meyer,⁴ championed in this country by Novak, for his invaluable demonstration of epithelial heterotopy and metaplasia, which helps to render more intelligible the occurrence of many of the extrapelvic growths of like character. Suffice it to say that at present two views are generally

From the Department of Gynecology, School of Medicine, Tulane University

1 Cullen, T. S. *Adenomyomata of the Uterus*, Philadelphia, W. B. Saunders Company, 1908

2 Ivanoff, N. S. *Monatschr f Geburtsh u Gynak* 7 295, 1898

3 Sampson, J. A. *Arch Surg* 3 245, 1921

4 Meyer, R., and Kitai, I. *Zentralbl f Gynak* 48 2449, 1924

held as to the cause of these tumors either Sampson's hypothesis of cellular spill or the theory of heteroplasia of a serosal cell, advocated as early as 1898 by Ivanoff. However, that neither of these theories can explain the occurrence of endometrioma at all the known sites is generally accepted. The theory of heteroplasia of the serosal cell cannot explain the perineal endometrioma, while endometriosis of the inguinal canal is difficult to ascribe to the theory of the implantation of fragments of the uterine lining.

It is not the intention in this paper to enter this field of discussion, and the following remarks and observations will hold good irrespective of the primary source of the endometrial elements. If the endometrioma is explained by the theory of implantation, there must be some stimulant or environment which determines whether the endometrioma will grow or perish. Sampson⁵ took cognizance of this fact and stated that "the escaping cells need suitable soil." If, on the other hand, the origin of the endometrioma is from heteroplasia of serosal or germinal cells, some factor must be present which first initiates the cellular change and subsequently stimulates the endometrial growth. The actual origin of the endometrioma is unimportant, whether from implantation of a living graft or from cellular metaplasia, the all-important question is the determination of the cause of the igniting factor which controls the cellular change or which stimulates the endometrial implant to proliferation.

The hypothesis on which this paper is based is that the fundamental igniting cause has its origin in the excessive stimulation of the aberrant tissue by the ovarian follicular hormone. That the action of this hormone is not confined to the uterine endometrium alone, as demonstrated by the endometrial changes during the normal menstrual cycle, but influences the genital tract as a whole is easily proved.⁶ When this hormonal action on the endometrium is abnormal, however, causing endometrial hyperplasia, it is equally abnormal in its action on ectopic endometrial tissue and causes, by means of cellular metaplasia of the potential serosal cells or by tumor proliferation of an aberrant endometrial implant, the formation of an endometrioma.

Through the work of Schroeder⁷ and Meyer⁸ in Germany, Shaw⁹ in England and Graves,¹⁰ Fluhman,¹¹ Novak,¹² Martzloff¹³ and

5 Sampson, J. A. *Surg., Gynec. & Obst.* **38** 287, 1924.

6 Estrogenic Substances. Theelin, report of the Council on Pharmacy and Chemistry, *J. A. M. A.* **100** 1331, 1933.

7 Schroeder, R. *Arch. f. Gynak.* **27** 102, 1915.

8 Meyer, R. *Arch. f. Gynak.* **93** 259, 1920.

9 Shaw, W. *J. Obst. & Gynec. Brit. Emp.* **36** 1, 1929.

10 Graves, W. P. *Am. J. Obst. & Gynec.* **20** 500, 1930.

11 Fluhman, C. F. *Surg., Gynec. & Obst.* **52** 1051, 1931.

12 Novak, E., and Martzloff, K. H. *Am. J. Obst. & Gynec.* **8** 385, 1924.

13 Martzloff, K. H. *Northwest Med.* **33** 263, 1934.

Burch¹⁴ in this country, the cause of endometrial hyperplasia has been thoroughly investigated. That the persistent and excessive stimulation of the ovarian follicular hormone, in the absence of any corpora lutea and with a possible anterior hypophyseal action in the background, is the cause of endometrial hyperplasia has been established by these observers. Burch^{14a} and his co-workers produced endometrial hyperplasia in spayed mice by injection of the estrogenic principle, while Hofbauer,¹⁵ by implantation of the substances of the anterior lobe or by injection of anterior hypophyseal extracts, demonstrated endometrial changes similar to those of endometrial hyperplasia. The general acceptance is at present that endometrial hyperplasia is the result of the unopposed and continued action of an excessive amount of the estrogenic principle derived from the multiple follicular cysts of the ovary.

The morphologic and functional characteristics of an endometrioma and of the uterine endometrium are similar, the integrity and function of the endometrioma are dependent on the presence of active ovarian tissue, since castration causes regression of the tumor, the endometrioma presents decidual reaction during pregnancy, it undergoes the phases of the menstrual cycle, changes dependent on the ovarian hormones, and, as proved by Gleave¹⁶ in rabbits, the presence of the estrogenic principle is essential for the maintenance of the lesion.

Since the ovarian follicular hormone is the cause of endometrial hyperplasia and since the histologic structure of the endometrioma and that of the uterine endometrium are similar, it is logical to deduce that the igniting factor of endometrioma which brings about the cellular metaplasia or the proliferation of the endometrial implant is the estrogenic principle. That such is the case is all the more firmly established by the fact that in many instances the endometrioma presents hyperplasia which is typical histologically of endometrial hyperplasia and also by the high incidence of the association of endometrioma with uterine endometrial hyperplasia. The frequent finding of all the features of endometrial hyperplasia in an endometrioma, accompanied with similar changes in the uterine mucosa, indicates that the endometrioma can be caused only by the factor which determines the mucosal changes—the ovarian follicular hormone.

A distinction between endometrial hypertrophy or hyperplasia and the formation of a true tumor should be made. In hypertrophy or hyperplasia the etiologic factor is an abnormally high concentration of the ovarian follicular hormone in the circulation, acting apparently on

14 Burch, J. C., et al. (a) *Surg., Gynec. & Obst.* **53** 338, 1931, (b) *Arch. Path.* **17** 799, 1934.

15 Hofbauer, J. *Surg., Gynec. & Obst.* **52** 222, 1931.

16 Gleave, H. H. *J. Path. & Bact.* **33** 675, 1930.

normally susceptible tissue. On the other hand, in the formation of a tumor, as in a case of endometrioma, the increased amount of the estrogenic principle in the blood acts on a hypersusceptible tissue which has the capacity to concentrate the hormone at the site of the tumor and to react by proliferation.

In a former contribution¹⁷ on the relationship between endometrial hyperplasia and uterine fibroids, the high incidence of ovarian endometrial implants (30 per cent) was noted. At that time the suggestion was made that these three conditions might possibly have a common etiologic background. These figures have been rechecked and combined with recent observations, and it has been found that ovarian and uterine endometriomas were associated with endometrial hyperplasia and uterine fibroids in 64 per cent of the 44 cases studied, a figure far too high to indicate a mere coincidence.

Analysis of 44 Cases of Endometriosis

Type of Operation	No. of Cases
Hysterectomy	44
Bilateral salpingo-oophorectomy	18*
Removal of all remaining ovarian tissue	10*
Unilateral salpingo-oophorectomy	16
Condition of the Myometrium	
Fibromyomatous	44
Hyperplastic	24
Fibrotic	6
Adenomyomatous	8
Normal	15
Condition of the Endometrium	
Hyperplastic	40
Premenstrual	4
Condition of the Ovaries	
Multiple follicular cysts	44
Corpus luteum (mature)	4
Miscellaneous	
Salpingitis	18
Adhesions	20
Ovarian endometrial transplants	22†

* All ovarian tissue was removed in 28 cases (64 per cent).

† Fifty per cent of the total, in combination with the 8 adenomyomas, 64 per cent of the total.

Other authors likewise have called attention to the frequent association of endometrial hyperplasia, endometrioma and uterine fibroids. In

¹⁷ Witherspoon, J. T. Surg., Gynec. & Obst. **56** 1026, 1933, Endocrinology **18** 703, 1933.

Jeffcoate's¹⁸ series of 113 cases of endometriosis 79 women (71 per cent) presented endometrial hyperplasia, while 31 (28 per cent) presented uterine fibroids. Allen,¹⁹ in his study of endometrioma, was impressed with the high incidence of the association of this condition with menstrual irregularities due to endometrial hyperplasia (70 per cent) and uterine fibroids (41 per cent) and the prevalence of relative sterility (60 per cent). Smith,²⁰ in 159 cases of endometrioma, noted associated endometrial hyperplasia in 42 per cent and uterine fibroids in 52 per cent of the patients.

Not only the uterine and the aberrant endometrium is stimulated to hyperplasia and tumor proliferation by the estrogenic principle, but the uterine musculature also is affected.²¹ Because uterine fibroids are slow-growing tumors, the stimulation of the ovarian follicular hormone must be effective over a prolonged period in order that the formation of the fibroid can occur. Geschickter, Lewis and Hartman²² have gone a step further in describing the action of the estrogenic principle. Their conclusion seems to indicate that gynecomastia in the male and virginal hypertrophy and the formation of fibro-adenoma in the female breast are dependent on the pathologic variations in the action of the ovarian follicular hormone on the epithelium of the mammary ducts, and that prolonged and interrupted stimulation by the estrogenic principle rather than brief, high concentration is necessary for the production of these abnormal conditions of the breast.

It therefore seems logical to deduce that the multiple follicular cysts of the ovaries, in the absence of corpora lutea, which cause, through the action of the estrogenic principle, endometrial, uterine and mammary hyperplasia or hypertrophy, likewise cause endometriomas, uterine fibroids and mammary fibro-adenomas.

There are two clinical features—functional uterine hemorrhage and sterility—that are associated with endometrioma, and their occurrence is explainable by the hypothesis just stated. Menstrual irregularities frequently accompany endometrioma, yet the source of the bleeding cannot, in many cases, be ascribed to the endometrial tumor. However, such hemorrhage can easily be explained by the associated endometrial hyperplasia of the uterine mucosa. Likewise the high frequency of sterility (60 per cent)¹⁹ with endometrioma is due to the presence of the multiple follicular cysts of the ovaries in the absence of ovulation and of the formation of corpora lutea.

18 Jeffcoate, T. N. A. *J. Obst. & Gynec. Brit. Emp.* **41** 684, 1934.

19 Allen, E. *Am. J. Obst. & Gynec.* **26** 803, 1933.

20 Smith, G. V. *Am. J. Obst. & Gynec.* **27** 806, 1929.

21 Witherspoon, J. T. *Surg., Gynec. & Obst.* **57** 87, 1934, footnote 17.

22 Geschickter, C. F., Lewis, D., and Hartman, C. G. *Am. J. Cancer* **21**

CONCLUSION

The hypothesis that all forms of overgrowth (hyperplasia, hypertrophy or tumor proliferation) of the uterine endometrium and musculature, of the aberrant endometrioma and of the mammary glands are due to the same factor—the estrogenic principle—not only is supported by clinical and pathologic data but it explains satisfactorily the simultaneous development of these conditions and their associated clinical features—uterine hemorrhage and sterility

TRANSPLANTATION OF SKIN AND CARTILAGE IN CHICKENS

LEO LOEB, M D
AND
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ST LOUIS

In a series of investigations we studied the transplantation of mammalian tissues, for this purpose, in the majority of cases, we used thyroid gland and cartilage with the adjoining tissues parathyroid gland and fat, bone and muscle tissues. Our principal aim in these experiments was to analyze the relations which might exist between the organismal differentials of the host and transplant and the mode and intensity of the reactions of certain host cells against the transplant, as well as to ascertain the degree of toxicity of the body fluids of the host for the grafted tissues. Definite relationships were observed to obtain between these two sets of conditions the character of the organismal differentials of the host and transplant and the effects of the host on the graft. We thought it of interest to extend these investigations to birds, in order to determine whether we should find here, also, similar connections between these two factors. Some experiments concerning transplantation of normal tissues in adult birds had already been made by several investigators (Loeb and Addison,¹ Schultz,² Danforth³), but these seemed as yet insufficient to allow us to arrive at definite conclusions so far as the problem stated here was concerned.

In regard to the technic to be applied in such investigations in birds and particularly in chickens, we suffered from a rather serious disadvantage as compared with one who undertakes similar experiments in mammals such as guinea-pigs, rats and mice. In the latter, one can readily use the aforementioned organs, which in their totality represent a sufficiently varied selection of tissues with which to judge the mode of reactions of the host against the transplant. In chickens, on the other hand, transplantation of the thyroid gland, on account of its situation

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1 Loeb, Leo, and Addison, W H F Arch f Entwcklgsmechn d Organ **32** 44, 1911

2 Schultz, W Arch f Entwcklgsmechn d Organ **35** 484, 1912, **36** 353, 1913

3 Danforth, C H, and Foster, F J Exper Zool **29** 52 and 443, 1928

far down in the neck adjoining the thorax, is much more difficult to accomplish without killing the animal, we chose, therefore, as tissues to be transplanted, pieces of skin in addition to the xiphoid cartilage and adjoining tissues. However, in using this technic, we introduced a number of variable factors which complicated the results and rendered their interpretation less certain. Even the fate of autotransplanted skin in chicken may vary considerably because the unavoidable transfer of feathers with the epidermis may injure the transplant to a different degree in different cases, furthermore, the results may vary in accordance with the thickness of the connective tissue on which the skin is situated, and there are other changes brought about by the way in which the surrounding host connective tissue may affect the ultimate shape of the grafted skin. In transplantation of mammalian skin a cyst is readily produced, lined on the inside by the transplanted epidermis and filled with masses of keratin. In the case of bird skin, a typical cyst occurs less readily, the skin is usually folded on itself without the typical formation of a cyst, although an imperfect cyst may form. In other cases, however, the epidermis may be everted so that the epidermis lines the outer instead of the inner side of this structure, and thus a nonspecific injury of the transplant tends to occur. Moreover, the epidermis as such, being a less sensitive reagent than the thyroid gland, is a less suitable indicator of the reaction of the host against the graft. As far as the xiphoid cartilage is concerned, the variety of associated tissues transplanted with it is usually less in birds than in mammals. In the majority of cases, this graft contains merely cartilage tissue with some adjoining dense tendon-like structures consisting of parallel strands of very dense fibrous tissue separated by rifts, in which the cells are situated.

There is a last difficulty which one has to face in such experiments in birds. Here the lymphocytes dominate in the circulation to a much greater extent than in mammals, the reaction of the host against the graft, a reaction in which, as we have shown previously, the lymphocytes play a dominating part, tends, therefore, to become very intense even when the discordances of either specific or nonspecific character are relatively slight, and this fact makes accurate grading of the degree of the reaction difficult. On the other hand, it is possible by means of transplantations in birds to study certain activities of lymphocytes more readily than by means of mammalian transplantations.

EXPERIMENTAL OBSERVATIONS

Series 1—We first studied autotransplantation. Examination took place between five and thirty-five days after the transplantation of the tissue, altogether fifty-one pieces of tissue were studied microscopically. As early as from five to seven days after the transplantation of skin, giant cells were found around the keratin occasionally, and connective tissue surrounded either the epidermis or the keratin. The epidermis of the transplants showed variable degrees of preserva-

tion, it was preserved in some cases and partly or entirely destroyed in others. When the transplanted epidermis and cutis were destroyed, the growing connective tissue of the host moved toward the keratin, and here cells situated in the connective tissue formed epithelioid and giant cells around the keratin. A connective tissue capsule often encircled the keratin masses with the giant cells. In other cases the transplanted epidermis was well preserved and formed a cyst in the center of which was keratin. In the preserved epidermal cells mitoses occurred, and, as a rule, the epidermis was best preserved when it rested on the transplanted cutis. Either collections of lymphocytes were lacking, or very small clumps of lymphocytes arranged around vessels could be seen. They did not penetrate into the epidermis. If keratinized material had been carried into the connective tissue, a few lymphocytes collected around it. Cartilage and the surrounding tendon-like connective tissue were usually well preserved, but occasionally some small areas of necrosis occurred in either or both of them. Lymphocytes were lacking altogether, or only very small collections formed around particles of fat tissue transplanted with the cartilage or around some foreign body. These lymphocytes usually were arranged around vessels, they were apparently not attracted by the cartilage or tendon-like tissue, as such.

Sometimes there took place under these conditions a disequilibrium between the cartilage and the connective tissue. Cells from the surrounding connective tissue or from the tendon-like tissue moved toward the piece of cartilage and collected around it. Connective tissue cells moved around the piece of cartilage in a concentric direction and formed a capsule about it. They often penetrated also into the periphery of the graft in a concentric direction corresponding to the fibrillar structure of the cartilage as indicated by the long axes of the cartilage cells. In some cases they then turned in a direction approximately at right angles to the long axis of the cartilage cells, thus penetrating slightly into the interior of the cartilage. Moreover, they possessed the power to split and dissolve the cartilage, in doing so, they sometimes enlarged. Either in the cartilage or in the surrounding dense fibrous tissue, some cells coming from the connective tissue could change into epithelioid or giant cells, especially in places where an obstacle presented itself to their progress. The connective tissue cells also accompanied some vessels which grew into the cartilage. It seemed that the connective tissue was especially attracted by large cell epiphyseal cartilage. No mitoses were observed either in the connective tissue or in the cartilage cells. In some instances, also, transplanted fat tissue and bone marrow with myelocytes were well preserved.

We found then, on the whole, that the transplanted cartilage was well preserved, and in no case did we find extensive collections of lymphocytes around it. On the other hand, active migration on the part of the connective tissue was often noticeable, and giant cells developed around hard and rough material.

Series 2, 3 and 4—We shall now compare with the results of autotransplantations those obtained in a series of syngenesiotransplantations (series 2) in which pieces were transplanted from brother to brother (or sister), in some of these experiments strains of chickens that had been inbred for a number of years were used. Altogether seventy-nine pieces of skin and cartilage were transplanted, and the examination took place from five to thirty-five days after the transplantation. In series 3, the same kinds of tissues were transplanted into other, not near-related chickens belonging to the same variety, such as White Leghorn or Rhode Island Red chickens. As a rule, tissues were exchanged between birds belonging to two strains of the same variety obtained from different breeders. In some of these experiments long inbred strains were used. Here, twenty-nine transplants of skin and the same number of transplants of cartilage, totaling fifty-eight pieces, were

examined at periods varying between nine and thirty-five days following transplantation. In series 4, transplantations were carried out between birds belonging to different varieties, such as White Leghorn, Plymouth Rock and Rhode Island Red. Forty-six pieces of skin and fifty-one pieces of cartilage, making altogether ninety-seven pieces, were examined from eight to thirty-five days after transplantation. We shall not give a complete survey of our findings, but shall report on some observations made at certain periods following transplantation.

(a) From the Fifth to the Ninth Day Following Transplantation. Series 2. As early as after five days, small masses of lymphocytes were found around some capillaries. Occasionally collections of lymphocytes were somewhat denser. During the eighth and ninth days, lymphocytes moved into the fibrous tissue and began to dissolve it. They were found also in the fibrous tissue underneath the epidermis in the skin transplants. They penetrated into the tendon-like transplanted fibrous tissue, primarily along the preformed longitudinal interstices, which offered a path of least resistance. In some collections of lymphocytes small particles of detached fibrous tissue of the host were found, which we may take as an indication of the destructive action of the lymphocytes. Connective tissue cells, enlarged and with ameboid processes, invaded the peripheral parts of the cartilage and the necrotic bone. The transplants were surrounded by connective tissue capsules, which also sometimes contained a few strands of lymphocytes.

Series 3 and 4. The findings were similar, the lymphocytic infiltration on the whole was slight, although it was possibly a little more extensive and denser than in series 2.

Series 4. Some lymphocytes invaded the epidermis and the tendon-like fibrous tissue. Giant cells were found around the keratin, especially where the epidermis was missing. The latter seems to exert a certain protective effect against the invasion of the transplant by the connective tissue of the host.

(b) From the Tenth to the Eleventh Day Following Transplantation. Series 2. The lymphocytic infiltration on the whole was not very extensive, although in some places it may have been more marked, some lymphocytes penetrated into the transplanted epidermis.

Series 3. The tendon-like fibrous tissue was invaded by enormous masses of lymphocytes. The lymphocytes accumulated especially around blood vessels and united into nodes. They invaded the epidermis, a part of which they destroyed. Connective tissue cells, as well as nodes of lymphocytes, invaded also the tendon-like fibrous tissue, the lymphocytes as usual moving especially in the preformed longitudinal interstices, the lymphocytes entered also cartilage and bone and destroyed both tissues. Some giant cells formed around necrotic areas of cartilage. Although the lymphocytic infiltration was marked, it affected as yet mainly the connective tissue around the transplants and to a less extent the transplanted tissues themselves.

Series 4. A system of lymphocytic nodes had developed around vessels. The conditions here were similar to those seen in series 3.

(c) From the Twelfth to the Thirteenth Day Following Transplantation. Series 2. After thirteen days we found for the first time in syngenesiotransplants dense masses of lymphocytes in the connective tissue surrounding the transplants, also nodes of lymphocytes directly around the cartilage, as well as tendon-like fibrous tissue around the epidermis. Within some of the lymphocytic nodes there were seen remnants of muscle fibers and detached pieces of fibrous tissue, which also may be taken as an indication of the destructive action of the lymphocytes, these cells also entered the epidermis and broke into the keratin. In the dense fibrous tissue they moved with ameboid processes in the direction of the fibers.

The transplanted muscle tissue was necrotic, the transplanted dense tendon-like fibrous tissue also contained some necrotic areas

Series 3 Here, too, dense masses of lymphocytes, partly in the form of distinct nodes, surrounded the transplant including the cartilage. Lymphocytes penetrated also into living epidermis and injured it

Series 4 Again, dense masses of lymphocytes were found, not only surrounding the cartilage, but penetrating into it and replacing a great part of this tissue. While the dense fibrous tissue inhibited and retarded to some extent the advance of the lymphocytic masses into the transplant, the lymphocytes succeeded in boring their way also into this fibrous tissue, and in the end these cells in compact masses invaded and then dissolved it, even if its cells were still alive. Lymphocytes and also connective tissue cells, in their destructive action, detached pieces of cartilage and bone from the transplant. In the cartilage, the lymphocytes opened the spaces in which the cartilage cells were lying and filled these cavities. Also around the skin transplant there were in places very dense infiltrations resembling lymph nodes, yet in the transplanted epidermis mitoses could still be seen, and great accumulations of lymphocytes separated the epidermis from the surrounding connective tissue. We then noticed for the first time collections of lymphoblasts in the peripheral parts of these nodes. The lymphocytes as a rule accumulated first around blood vessels. While in the majority of these specimens there was thus a very marked lymphocytic infiltration, it was not present in all transplants, some variations occurring in this respect

(d) From the Twenty-Fifth to the Twenty-Eighth Day Following Transplantation Series 2 Cartilage and tendon-like fibrous tissue were found to a variable degree dissolved and replaced by nodes of lymphocytes, the peripheries of which were in certain cases surrounded by lymphoblasts, in other instances lymphoblasts themselves formed follicles situated in the peripheries of the lymphocytic infiltrations. Mitoses occurred in these lymphoblasts, occasionally hemorrhages were seen in the nodules. Also lymphoblasts were able to invade and destroy the tendon-like tissue. Lymphocytic nodes surrounded the keratin of the skin transplants. Connective tissue in places grew into necrotic areas of cartilage, leading to the development of fibrillar connective tissue

Series 3 Necrotic tissue, cartilage and tendon-like connective tissue were surrounded by a number of lymphocytic nodes, from these nodes lymphocytes penetrated into the cartilage, which they dissolved, and, furthermore, into the tendon-like tissue in the direction of the preformed interstices. Likewise connective tissue cells penetrated into the cartilage and also into bone and dissolved these tissues

Series 4 Enormous masses of lymphocytes were found around the various tissues of the transplants. They formed a continuous ring of lymphocytic nodes about these structures, penetrating into them in strands and, in the end, overwhelming and destroying them. The cartilage was also invaded by these cells, preferably in the direction of the fibrillae in the cartilage, it was evidently easier for them to proceed in these interstices than to push their way into the cartilage at right angles to this direction. They were furthermore able to convert the tendon-like tissue into a finely fibrillar material which stained much more lightly with eosin than the original tissue which they had invaded. Some lymphocytes enlarged while entering the cartilage. In certain instances bone also was overwhelmed with lymphocytes, and lymphocytes were seen between the keratin material, which, in addition, was surrounded by hyaline masses corresponding to necrotic giant cells. Connective tissue cells likewise invaded the cartilage and here assumed the character of a myxoid connective tissue

COMMENT AND CONCLUSIONS

From these experiments we may draw the following conclusions. In birds, as well as in other classes of vertebrates, the relation of the organismal (individuality, race) differentials is the essential factor which determines whether or not the host will affect the transplant injuriously. In autotransplantations injurious reactions are lacking, except those which are due to extraneous accidental factors, while in all other transplantations (syngenesiotransplantations, homeotransplantations and interracial transplantations) they are present. They were also found in former experiments in which homeotransplantation and heterotransplantation of skin was carried out in birds.

In birds the lymphocytes represent a much higher percentage of the leukocytes circulating in the blood than in mammals, correspondingly in birds the lymphocytic reaction against a transplant is much more intense than in mammals if the organismal differentials of the host and the transplant differ. Thus even after syngenesiotransplantation in inbred strains of fowls the lymphocytic reaction may be very intense. This great intensity of reaction and the great sensibility of birds to strange transplants, resulting from the great activity of the lymphocytes, make difficult a finer gradation of the intensity of the reaction in accordance with the relationship between the host and the transplant, as difficult as would be an attempt to measure differences in weight as great as many grams by using a balance responding to differences of a fraction of a milligram. Nevertheless, there are indications that, on the average, the reactions were least intense in transplantations between brothers belonging to inbred strains and most intense in cases of interracial transplantation. However, since in the former type in individual instances there occur certain variations in the strength of reaction, which sometimes can be very strong, it would be difficult to prove such a gradation in an exact manner, although there is, as stated, an indication that a relation between the intensity of reaction and the distance of relationship between the organismal differentials exists.

Because of the great intensity of the lymphocytic reaction in birds against transplants of chicken tissues, especially those of cartilage and tendon-like fibrous tissue and even bone, it is possible to recognize the marked ability of lymphocytes to invade, destroy and, in particular, to dissolve resistant tissues like cartilage and dense fibrous tissue. In homeotransplantations and interracial transplantations (series 3 and 4) extensive lymphocytic infiltration was first observed after ten and eleven days, while in syngenesiotransplantation (series 2) it was first observed after thirteen days. The lymphocytes either dissolve such grafts or leave merely a finely fibrillar material, in some cases their destructive action may detach pieces of fibrous and of muscle tissue, which are then

included in nodular accumulations of these cells. The lymphocytes invade the transplant preferably in the direction of least resistance, thus the pseudopods, which the lymphocytes as well as connective tissue cells send out while migrating, have evidently the best chance of effecting locomotion of the whole cell. However, lymphocytes are able to penetrate also at right angles to these preformed fissures and to invade the tissue directly. The same observation can be made in the case of connective tissue cells, but it seems that while, as a rule, connective tissue cells invade only dead tissue, lymphocytes more readily invade and destroy living structures. However, connective tissue cells are able to push their way between living structures also and thus to separate them.

The reaction of lymphocytes against transplants leads to the accumulation of these cells around blood vessels. Here they form follicle-like masses, but toward the end of the second week or during the third week there appear, in addition to the small lymphocytes, collections of large lymphoblast-like cells, in which mitoses are frequent. These cells may collect also in the form of small follicles, situated in a diffuse mass of lymphocytes, especially in the periphery of the connective tissue and surrounding the transplants, and they may also invade and destroy the strange tissue. Such lymphoblastic accumulations remained visible until the end of the period during which we followed the fate of the transplants, they were found in syngenesiotransplantations as well as in homeotransplantations and interracial transplantations, but around interracial transplants collections of lymphoblast-like cells appeared first in the peripheral parts of the lymphocytic nodes, about thirteen days after transplantation, while lymphoblasts with mitoses appeared in the second series at sixteen days.

We may therefore conclude that birds react quite generally in a very intense manner against the presence of tissue possessing strange organismal differentials. Danforth and Foster³ apparently arrived at a different conclusion. They transplanted skin flaps from recently hatched chicks either to other chicks of the same inbred races or to other races, such as White Leghorn and Plymouth Rock. In many cases the pieces of skin healed in permanently in chickens belonging to other races, although the best results were obtained in interchange of skin between members of the same inbred race, but this may have been due to accidental factors rather than to a similarity of the organismal differentials between the host and the transplant. These authors stated their belief that individuality differentials exist in birds in isolated instances.

However, this interpretation of their results is not the only one possible. In the first place, we must consider that they used for their transplantations recently hatched chicks rather than adult birds, and judging from experiences with mammals and also with birds, we should expect the reactions in very young hosts to be much milder. It is furthermore

possible that when skin grafts heal in, in very young hosts, a gradual adaptation may take place between the host and the transplant so that the grafts remain attached to the host even after the animal has reached the adult stage. Secondly, the "take" or "nontake" of a transplant is not a fully satisfactory test for the degree of incompatibility of two organismal differentials. A much finer index consists in the measurement of the intensity of the reaction of the lymphocytes, connective tissue and blood vessels of the host against the transplant. Using such tests, we were able in these experiments to prove that the individuality differentials even between brothers belonging to inbred strains may differ from each other, and that homeotransplantation and interracial transplantation lead to very severe reactions of the host against the transplant. Our results also accord with the findings of May,⁴ who observed that in adult lizards homeotransplantation of skin does not succeed, whereas autotransplantation is successful.

SUMMARY

Organismal and, in particular, individuality differentials are present in birds, as well as in mammals, this is proved by the difference in the lymphocytic reactions after autotransplantation and after syngenesio-transplantation and homeotransplantation. However, even relatively small differences in the constitution of the organismal differentials cause so marked a lymphocytic reaction in birds that quantitative determinations of differences in the organismal differentials between different individuals in this species are rendered difficult.

In birds the activity of lymphocytes and lymphoblasts is so intense in transplantations other than autotransplantation that the destructive effect, which both small and large lymphocytes exhibit, can be readily recognized. This destructive effect applies even to cartilage and bone substance.

⁴ May, R. M. *Brit J Exper Biol* **1** 539, 1924. *Transplantation animale*, Paris, Gauthier-Villars, 1932.

SERPENTINE ANEURYSM OF THE INTERNAL CAROTID ARTERY

WITH RESULTING ENCEPHALOMALACIA AND CEREBRAL HEMORRHAGE

OTTO SAPHIR, M D

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In a previous communication¹ anomalies of the circle of Willis were discussed which were thought to play an important rôle in the causation of encephalomalacia and cerebral hemorrhage. It was pointed out that there is an increasing tendency to attribute to functional abnormalities some anatomic changes which are undoubtedly the result of vascular lesions. This is particularly true in certain instances of encephalomalacia and cerebral hemorrhage. For this reason, as was stated before, every demonstrable morphologic cause of vascular disturbance should be searched for, carefully evaluated and ruled out before an explanation is given based on functional disturbance. Anomalies of the circle of Willis with resulting interruption of the circulation between the internal carotid and the vertebral arteries may, under certain circumstances, form the anatomic basis for encephalomalacia and cerebral hemorrhage.

The purpose in this communication is to describe three instances of encephalomalacia and cerebral hemorrhage, the causes of which could not be determined until the internal carotid arteries were completely dissected and severe occluding lesions found in them. These lesions were thought to have been the etiologic factor in the lesions of the brain.

REPORT OF CASES

CASE 1—A 75 year old woman who had shown moderate senile dementia for the previous eleven years was found unconscious at home the day she was admitted to the hospital. On examination there were Cheyne-Stokes breathing and right-sided hemiplegia. The pupils were contracted and did not react to light. There were moist râles on the right side of the chest, the heart tones were faint. The face and the lower extremities were edematous. At the time of her admission to the hospital the rectal temperature was normal, but in the next five hours it rose to 101.6 F. The white blood cell count was 24,700, with 90 per cent polymorphonuclear leukocytes. The arterial blood pressure was 144 systolic and 80 diastolic. The clinical impression was that she had suffered a cerebral hemorrhage of the left hemisphere involving the internal capsule. The eyegrounds were not examined.

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1 Saphir, O. Anomalies of the Circle of Willis with Resulting Encephalomalacia and Cerebral Hemorrhage, *Am J Path*, to be published.

At autopsy, the heart weighed 325 Gm. There were coronary arteriosclerosis, myocardial fibrosis and chronic passive hyperemia of the various organs. There was a generalized arteriosclerosis, with marked thickening of the arteries at the base of the brain. No occlusion of the lumens of these vessels could be demonstrated. There were nephrosclerosis of the arterial variety and bilateral confluent bronchopneumonia. The skull revealed no evidence of fracture. There was a cerebral hemorrhage involving the left capsula extrema, claustrum, external capsule and putamen and the posterior region of the left internal capsule. The adjacent white substance of the brain up to the anterior portion of the left parietal lobe was grayish pink and finely granular and did not reveal the normal architecture. The left lateral ventricle was filled with clotted blood.

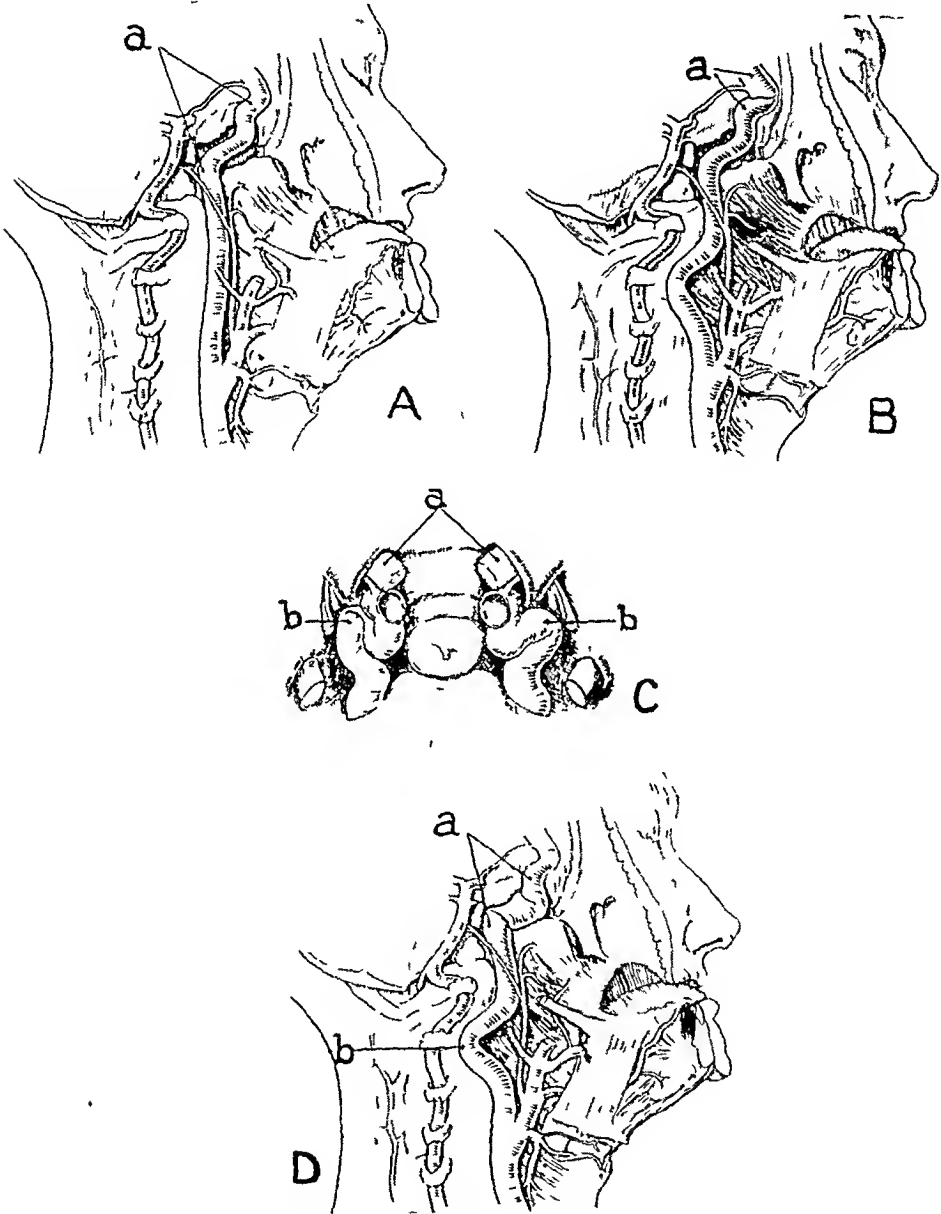
The cerebral arteries were dissected, but neither they nor their finer branches showed occluding lesions. The vertebral arteries were dissected next but showed no occluding lesion, though arteriosclerotic plaques were numerous. Since no definite explanation of the cerebral hemorrhages and encephalomalacia could be given, the possibility that a functional abnormality in the involved vessels had caused these conditions was seriously considered. However, before this explanation was accepted, both internal carotid arteries were completely and carefully dissected. These arteries, in the posterolateral regions of the pharynx, showed distinct tortuosity, the lumens of the arteries were slightly encroached on. In the region of the cavernous sinus, on each side, a firm, tumor-like mass was palpated. When the structures of the cavernous sinuses were dissected, it was found that what had seemed, on palpation, to be tumors were markedly rigid internal carotid arteries. Each vessel, from the intracranial opening of the carotid canal to the point where it divides into its final ramifications, was markedly elongated and had assumed the form of an S with very sharp curves. The curves closely approximated one another. When the left artery was opened, it was seen that at the middle curvature of the S-like loop a ridge was formed which completely occluded the lumen. The ridge consisted of a thin calcified plaque. A similar ridge was found projecting into the lumen of the right carotid artery at the corresponding site. This artery, however, though its lumen was extensively encroached on, was still patent.

Histologic examination of the brain revealed in many fields only indistinct outlines of brain tissue with many red blood corpuscles free in the tissue and many scavenger cells. Sections taken from the internal carotid artery in the region of the cavernous sinus showed areas of hyalinization and calcification and large atheromatous cavities.

Summary—The brain of a 75 year old patient who was found unconscious with right hemiplegia showed large areas of hemorrhage and encephalomalacia. Each internal carotid artery showed tortuosity in the region of the neck and elongation with S-like curvatures in the region of the cavernous sinus. In the latter region the left artery revealed complete occlusion of its lumen, while the lumen of the right artery was markedly narrowed.

CASE 2—A white woman, 60 years of age, who had had diabetes mellitus for a number of years, was admitted to the hospital because of pains in and discoloration of the right leg and toes. On physical examination the heart revealed a systolic murmur at the apex and at the base. The arterial blood pressure was 170 systolic and 62 diastolic. The plantar surface of the left foot revealed early gangrene. The urine contained dextrose (4+), acetone (4+) and diacetic acid (3+), and the blood contained 162 mg. of dextrose per hundred cubic centimeters. Five days before death, right-sided hemiplegia without coma and motor aphasia developed. The patient finally contracted bronchopneumonia, from which she died.

Autopsy revealed bilateral bronchopneumonia, generalized arteriosclerosis, coronary arteriosclerosis, myocardial fibrosis and chronic passive hyperemia of the various organs. Medial calcification of the femoral, popliteal and anterior and posterior tibial arteries was found, with occlusion of the lumens in various places. The left foot and the toes of the right foot were gangrenous. The brain revealed



Serpentine aneurysms of the internal carotid artery. *A*, the course of the carotid artery, the petrous portion of the artery is shown at *a*. *B*, carotid artery in case 1, with a serpentine aneurysm in the region of the cavernous sinus, shown at *a*. *C*, optic nerves (*a*) and internal carotid arteries in the region of the cavernous sinus in case 1, seen from above (*b*). *D*, carotid artery in case 3, with a serpentine aneurysm in its petrous portion (*a*) and a tortuosity in the posterolateral region of the pharynx (*b*).

a large area of softening, dark gray and finely granular, which extended through the white substance of the left temporal lobe to involve portions of the left parietal and the adjacent portion of the occipital lobe. The arteries at the base of the brain showed marked arteriosclerosis. Each internal carotid artery, in the region of the cavernous sinus, was lengthened and revealed an S-like tortuosity. The lumen of the left internal carotid artery corresponding to the distal curvature of the S loop was occluded by a ridge which was formed by a portion of the inverted wall of the artery. The lumen of the right carotid artery was markedly narrowed. The branches of both internal carotid arteries were carefully dissected, and no occluding lesions were demonstrable.

Histologic examination of the brain revealed its architecture obscured in many fields. There was an extensive infiltration by scavenger cells, and in many sections these cells were seen surrounding small blood vessels. A few lymphocytes were also present. Other sections showed many red blood cells free in the tissue. Sections through the carotid artery revealed areas of hyalinization and calcification and atheromatous cavities.

Summary—The brain of a 60 year old woman in whom hemiplegia developed revealed encephalomalacia with areas of hemorrhage. On each side the internal carotid artery was markedly lengthened and tortuous in the region of the cavernous sinus. The lumen of this portion of the left artery was occluded.

CASE 3—A 70 year old woman suffered severe dizziness and gradually sank into coma on the morning of her admission to the hospital. She had been well prior to this, and her arterial blood pressure had never been high. On examination it was noted that the pupils did not react to light and were slightly irregular. No abnormalities of the heart were found. The pulse rate was 68 beats per minute, and the arterial blood pressure was 148 systolic and 80 diastolic. Coarse râles were heard in both lungs. The Babinski reflex was positive bilaterally. The eyegrounds could not be examined.

Autopsy revealed generalized arteriosclerosis with coronary arteriosclerosis, myocardial fibrosis and chronic passive hyperemia of the various organs. There were acute purulent bronchitis and bilateral bronchopneumonia. The skull presented no abnormalities. The subarachnoid space contained a moderate amount of hemorrhagic liquid. A large area of hemorrhage and encephalomalacia was found in the right frontal lobe and in the white substance of the right parietal lobe. The hemorrhage extended into the right lateral and third ventricles. The right capsula extrema, external capsule, claustrum and lenticulate nucleus were almost completely destroyed. The right internal capsule, however, was well preserved with the exception of its most lateral portion. The arteries at the base of the brain showed severe arteriosclerotic changes. Each internal carotid artery was carefully dissected throughout its whole course. There were distinct tortuosity and lengthening in the posterolateral regions close to the pharynx. The lumens in these regions were slightly narrowed. The most severe changes were found in the petrosal portion of each internal carotid artery, within the carotid canal. Here the arteries were almost completely transformed into pipelike structures. Both arteries were curved into S-like loops with production of ridges. That in the right artery had led to occlusion of the lumen. In the region of the cavernous sinus each artery was thickened but not elongated.

Histologic examination of the brain revealed large areas of hemorrhage with only remnants of brain tissue. In other areas the architecture of the brain could not be made out, the nuclei were poorly stained, and a few scavenger cells were observed.

Summary—The brain of a 70 year old woman who died in coma revealed hemorrhages and encephalomalacia. The internal carotid arteries in their cervical portions showed distinct tortuosity. In their petrosal portions these arteries were lengthened and tortuous, forming S-like curves with production of ridges, which caused marked narrowing of the lumen of the left, and occlusion of the lumen of the right, artery.

COMMENT

These three instances are significant in that they reveal severe occluding lesions in the internal carotid arteries at locations where such lesions as a rule are not looked for, namely, in the region of the cavernous sinus and within the carotid canal of the temporal bone. Narrowings of the lumens were also present in the first portions of the internal carotid arteries, in the pharyngeal region. The encroachment on the lumens was the result of the tortuosity of the vessels with inversion of parts of their walls and formation of bends or kinks.

The tortuosity of the arteries may be referred to as cirroid aneurysm, following the classification of Kaufmann² and of Karsner.³ Kaufmann stated that in instances of cirroid aneurysm the involved artery is dilated, elongated and tortuous. Karsner emphasized that the cirroid aneurysm is distinctly more common in the smaller vessels, such as the temporal arteries, where there is general dilatation associated with lengthening and tortuosity. In this form of aneurysm the primary condition is some variety of arteriosclerosis. Jores,⁴ on the other hand, stated that the dilatation of the lumen of an artery combined with tortuosity should be referred to as serpentine aneurysm. He reserved the term "cirroid aneurysm" for only those instances in which branches of the primarily involved artery are similarly dilated and tortuous. If Jores' classification is used, the lesions described here should be classified as serpentine aneurysms.

Tortuosity of the internal carotid artery in the posterolateral region of the pharynx is well described by Brown-Kelly,⁵ who mentioned all degrees of bending between extreme tortuosity and slight curvatures. Skillern⁶ described a marked degree of tortuosity of the artery in the cadaver of a person apparently 60 years old. The artery was uniform in caliber. Beginning at the skull and extending two thirds of the distance to

2 Kaufmann, E. *Lehrbuch der speziellen pathologischen Anatomie für Studierende und Ärzte*, ed 7 and 8, Berlin, W de Gruyter & Co., 1922.

3 Karsner, H. *Human Pathology*, Philadelphia, J B Lippincott Company, 1926.

4 Jores, L., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1924, vol 2, p 608.

5 Brown-Kelly, A. *J Laryng & Otol* 40 15, 1925.

6 Skillern, P G. *J A M A* 60 172, 1913.

the bifurcation of the common carotid artery, an S-shaped tortuosity was observed, the bends of which extended at no time more than 1 cm from a line representing the axis of the normal artery. Fisher⁷ stated that such tortuosity may be explained phylogenetically, since in many of the mammalia the internal carotid artery is even more tortuous than it is in man, and quoting Chauveau he stated that the internal carotid artery of the seal, for instance, is actually forty times as long as the distance it has to traverse. This, however, could not be confirmed by Carmel.⁸ Brown-Kelly described the tortuosity of the artery in persons of varying ages and stressed its relation to senile (arteriosclerotic) changes and to congenital malformations. Streit⁹ stated that arteriosclerosis was the principal cause of the tortuosity which he described in the internal carotid artery. Schaeffer¹⁰ reviewed the literature and stressed the fact that age has no bearing on the development and presence of tortuosity in an internal carotid artery. Carmack¹¹ stated that aberrations of the internal carotid arteries probably occur more frequently than is realized, and that their occurrence invariably brings the vessel closer to the tonsil and pharynx.

Jastram,¹² quoting Broesike, named five locations where curvatures of the internal carotid artery may predispose to tortuosities: (1) just behind the bifurcation, (2) just beneath the base of the skull, (3) within the carotid canal, (4) within the sulcus caroticus close to the sella turcica, (5) in the region of the impressio carotica of the sphenoid bone. It may be mentioned that no reference to tortuosity of an internal carotid artery within the carotid canal and cavernous sinus could be found in the literature studied.

It seems clear that in those regions where the internal carotid artery normally is slightly tortuous, this tortuosity may increase in arteriosclerosis. Jones called attention to the fact that the arteriosclerotic process is often characterized by dilatation of the involved blood vessels combined with tortuosity. In none of the three instances described, however, was the tortuosity of the internal carotid artery in the posterolateral region of the pharynx severe enough to have led to a marked narrowing or occlusion of its lumen.

The sigmoid tortuosity of the artery within the carotid canal of the temporal bone was much more significant, because it had led to the formation of a ridge which occluded the lumen. The space within the

7 Fisher, A. G. T. *Lancet* **2** 128, 1915

8 Carmel, A. G. *Anat. Rec.* **39** 343, 1928

9 Streit, H. *Ztschr. f. Hals-, Nasen- u. Ohrenh.* **30** 315, 1931

10 Schaeffer, J. P. *J. A. M. A.* **77** 14, 1921

11 Carmack, J. W. *Laryngoscope* **39** 707, 1929

12 Jastram, M. *Beitr. z. klin. Chir.* **93** 341, 1914

carotid canal is limited. The walls of the canal, being osseous, cannot expand when the artery, as a result of arteriosclerosis, tends to become wider and elongated. The inevitable result must be inversion of parts of the wall with formation of kinks and, because of the elongation, a change from the normal simple curve in this location to the S-like tortuosity. The walls of the carotid canal were still smooth. This indicates that the changes were not old enough to have produced erosions of the adjacent bony structures.

In the first two instances the occluding lesions of the internal carotid arteries were found in the region of the cavernous sinus. The sigmoid tortuosity in the first skull was so marked that it gave, on palpation, the impression of a tumor. And yet, grossly, little was seen until the dura was removed and the cavernous sinus was dissected. The increase in length of the carotid artery from where it entered the cranial cavity to the point at which it divided into its final branches was about three times the normal length in this area in the first instance, and about two and a half times the normal length in the second instance. The disproportion between the limited space in this region and the increase in length had led to the sigmoid tortuosity of the artery. The combined effect of the tortuosity and the widening of the lumen because of the arteriosclerosis had caused inversion of the wall at a point of the S-like curvature with formation of a ridge which resulted in the occlusion of the lumen.

As has been shown, an occluding lesion in the internal carotid artery was present in every instance. This lesion must be considered as at least one factor in the causation of the encephalomalacia and cerebral hemorrhages. The other factors were the arteriosclerosis of the arteries at the base of the brain and the coronary arteriosclerosis with resulting myocardial fibrosis which was found in every heart.

Salinger and Pearlman¹³ recently discussed ligation of the internal (common) carotid artery. They stressed the fact that in a number of patients one internal carotid artery can be ligated without causing vascular disturbances of the brain, because of the adequate supply through the circle of Willis. In senescence, however, with increasing arteriosclerosis of the arteries at the base of the brain and consequent narrowing of the lumens of the arteries it seems clear that the anastomoses cannot function as well as normally. And yet the circulation of the brain may be adequate to prevent vascular disturbances even though one internal carotid artery is occluded. In patients who have coronary arteriosclerosis with increasing myocardial fibrosis, however, the point finally will be reached at which the myocardium becomes insufficient, either gradually or suddenly. Hence, if the vis a tergo is reduced, there may occur vascular insults to the brains of those patients

13 Salinger, S., and Pearlman, S. J. *Arch Otolaryng* 19 464, 1933

who show lesions in the circle of Willis or in the internal carotid arteries or in both. It seems important to consider these three factors in every instance of encephalomalacia and cerebral hemorrhage in the absence of thrombi or emboli in the arteries of the brain.

The question arises why the entire hemisphere of the brain was not involved when one internal carotid artery was occluded and there was a considerable amount of arteriosclerosis in the vessels at the base of the brain. Because the vessels of the circle of Willis and the vertebral arteries were patent, there evidently was sufficient collateral circulation to prevent encephalomalacia being caused by the vascular changes as long as the heart maintained its normal function. When the myocardium began to fail the collateral circulation was apparently insufficient, and encephalomalacia and hemorrhage followed.

In evaluating the causes of vascular disturbances of the brain, the circle of Willis must be given careful consideration, as was brought out in the previous communication.¹ It was shown that anomalies of the circle of Willis exist which result in complete separation of the two main arterial channels of the brain. Such anomalies are encountered not infrequently in routine autopsies and per se are of no consequence. Two brains were described¹ which revealed areas of encephalomalacia and cerebral hemorrhage without occluding lesions in the supplying arteries. In both instances, however, the blood could not pass through the posterior communicating arteries, and there was also evidence of myocardial failure. In these two instances, one of the three factors mentioned was very pronounced, namely, the absence of the collateral supply through the circle of Willis. In the three cases reported here, another of the three factors occupied the paramount place, namely, arteriosclerosis of the internal carotid artery with occlusion of its lumen. As mentioned before, in every instance of this series evidence of myocardial failure was also found. This was based, first, on the myocardial fibrosis and, second, on the passive hyperemia of various organs. It may be especially emphasized that this conception of the pathogenesis of encephalomalacia and cerebral hemorrhage is based on morphologically demonstrable lesions and deductions drawn from these findings.

In this study I have attempted to show the importance of post-mortem examination of the entire body in the explanation of these brain changes, and particularly examination of the heart and internal carotid arteries, as well as of the brain. I have also attempted to show the importance of the examination of the various organs for evidence of chronic passive hyperemia. In every instance of vascular disturbance of the brain the entire course of the internal carotid arteries should be examined in order to locate morphologically demonstrable causes of

the brain lesions. An examination of both vertebral arteries may also prove to be of value. All these factors should be looked for and carefully evaluated or eliminated before the pathologist resorts to an explanation primarily based on functional disturbances.

The eternal reproach with which the morphologist is confronted culminates in the accusation that his reasonings are "post hoc." Naturally, it is the duty of the pathologist to combine the various observations at autopsy and to try to relate the changes to primary lesions. In trying to adhere to this principle, occasional misconceptions may occur and a cause and effect relationship may be attributed to lesions which are only coincidental. However, it seems preferable for the morphologist to err in this respect rather than to resort too quickly to explanations which are not based on anatomically demonstrable lesions. It is conceivable that in the instances reported here and in the previously reported instances in which brains revealed anomalies of the circle of Willis, spasms of the arteries of the brain could have been held responsible for the brain changes, had the entire internal carotid arteries not been examined and had not sufficient attention been given to the circle of Willis. But since these lesions were found in addition to evidence of myocardial failure it seems clear that they can easily be correlated with the encephalomalacia and cerebral hemorrhages in a cause and effect relation. Perhaps more detailed postmortem examinations will result in the finding of anatomic lesions similar in some respects to those described here that will explain anatomic changes elsewhere which now are attributed to functional disorders.

SUMMARY

In every instance of encephalomalacia and cerebral hemorrhage, the internal carotid and vertebral arteries should be carefully examined throughout their course in order to locate morphologically demonstrable causes of the lesions of the brain. Three procedures should be observed in the explanation of these lesions in the absence of occlusions of the vessels at the base of the brain or of their branches. 1 The internal carotid and vertebral arteries should be examined throughout their course for occluding lesions. 2 The state of the circle of Willis should be studied with particular reference to congenital anomalies which may have caused an interruption of its continuity. 3 Morphologic evidence of cardiac failure should be looked for, such as coronary arteriosclerosis with resultant myocardial fibrosis and chronic passive hyperemia of the various organs. All these anatomic lesions should be searched for and carefully evaluated or eliminated before the pathologist resorts to an explanation based primarily on functional disturbances. Three instances of encephalomalacia and

cerebral hemorrhage are reported. Occlusions of the internal carotid arteries were found at locations where such lesions as a rule are not looked for, namely, in the region of the cavernous sinus and within the carotid canal of the temporal bone. The occlusions were the result of the tortuosity of the arteriosclerotic vessels with inversion of the walls and formation of bends or kinks. If Jores' classification is used, such a lesion of the artery is referred to as serpentine aneurysm.

STUDIES ON SPONTANEOUS RECOVERY FROM PNEUMOCOCCIC INFECTION IN THE GUINEA-PIG

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The studies here reported relate to two questions 1 What interpretation in relation to resistance to infection may be placed on the local cellular reaction in the course of infection? 2 What significance in relation to infection may be placed on the phagocytic activity of leukocytes in the course of infection?

Since we wished to observe the differences occurring when infected animals die and when they recover spontaneously, it was necessary to permit the experimental infection to run its natural course, it was therefore possible to observe only a limited part of the reactions occurring in the infected animals

MATERIAL AND METHODS

In all experiments guinea-pigs were used, in practically all cases a strain of *Pneumococcus* type I was used. The virulence of this strain was maintained by repeated passages through mice so that it was lethal for mice in dilutions of 10^{-8} or 10^{-9} . The guinea-pigs were infected by intraperitoneal injections of eighteen to twenty-four hour serum dextrose broth cultures, usually 0.25 cc of an undiluted culture was administered, as it was found that a limited number of animals so infected survived. After infection, fluid was withdrawn from the peritoneal cavity at periods of thirty minutes, one hour, six or eight hours, twenty-four hours, two days and subsequently at twenty-four hour intervals, smears of the peritoneal exudate were made and stained with Wilson's stain (for counting cells and determining phagocytosis), and cultures of the fluid were made to determine the presence of the infecting organism. In a number of cases comparison was made of the peritoneal fluid studied by the stained smear with that studied by intravital staining, in practically all cases the percentage of mononuclears was higher in the intravital stained specimens, so that there were from 5 to 10 per cent more mononuclears counted in these. Since there was thus relatively small but fairly constant difference we retained the stained smear in counting cells. At least 200 cells were counted and in many cases from 500 to 900 cells, several portions of the smear were consistently examined. The phagocytosis was determined on the basis of the number of cells of each type containing bacteria and is therefore expressed as a percentage of phagocytosing cells. In addition, in a considerable number of cases, but not in all, blood cultures were taken during the course of infection.

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PNEUMOCOCCIC INFECTION IN THE GUINEA-PIG

Usually within thirty minutes and always within an hour after injection, the organisms had passed into the blood stream. The animals showed little evidence of illness within the first twelve hours, but by twenty-four hours they were listless, their hair was ruffled, and they ate and drank little. If they survived the first twenty-four or forty-eight hours they showed emaciation. The duration of the infection varied widely. Some animals (eleven) died within the first twenty-four hours, a considerable number (ten) survived for two, three or four days and a few (ten) for from five to ten days, only to die after these longer periods. A few (three) died after the tenth day. Eighteen survived the infection.

In all cases in which the survival was shorter than six or seven days the pneumococci were constantly demonstrable in the peritoneal fluid. When, however, the period of survival was longer than seven days, it often happened that a positive culture was not obtained from the peritoneal fluid for a day or for several days before death. In some cases even in the last culture taken before the death of the animal no pneumococci were cultivated, in other cases, however, the pneumococci, after being absent from cultures for a time, were demonstrable before the death of the animal. In those experiments in which the animals receiving injections survived, the organisms had usually disappeared from the cultures of the peritoneal fluid before or at about the fifth or sixth day and did not reappear. Since some animals died from twenty to thirty days after the injection of pneumococci in spite of the fact that the cultures of the peritoneal fluid had been negative for two days or more, it was essential to formulate criteria of recovery. The guinea-pigs which died never recovered from the emaciation which occurred during the acute stage of infection. Therefore, only animals which survived beyond thirty days after the injection and which regained their weight after the acute stage of the infection were classed as recovering animals.

THE CELLS IN THE NORMAL PERITONEAL FLUID

The cells normally present in the peritoneal cavity of the guinea-pig were studied by Gardner¹ with intravital stains. Our studies were, as stated, carried out by means of stained slides. In counts in twenty-four normal animals we found the percental distribution of the leukocytes to be: neutrophils, from none to 5, eosinophils, from 15 to 27, lymphocytes, from 8 to 19, and mononuclears, from 60 to 75.

CHANGES IN THE CELLULAR ELEMENTS OF THE PERITONEAL FLUID
UNDER THE INFLUENCE OF IRRITANTS

Effect of Nonliving Irritants—If any foreign material or other irritant is introduced into the peritoneal cavity (0.9 per cent sodium chloride solution, broth, aleuronat, silver nitrate solution), in the first six hours there will be a drop in the number of mononuclears, a relative rise in the number of lymphocytes and eosinophils and somewhat later a further fall in the number of mononuclears, a fall in that of lymphocytes and of eosinophils and an increase in number or an appearance of neutrophils. This early sequence of changes is constant and occurs as a response to the introduction into the peritoneal cavity of any irritant (including pathogenic bacteria).

Often at six hours the neutrophils have reached a maximum percentage varying between 58 and 90, and by twenty-four hours the percentage of these cells has

¹ Gardner, L. U. Proc Soc Exper Biol & Med **26** 690, 1928-1929

usually but not always begun to fall. The percentage of mononuclears is usually at the lowest at six hours and has begun to rise at twenty-four hours. Lymphocytes and eosinophils are frequently absent at six and at twenty-four hours, or may be present only in very small numbers. At forty-eight hours the neutrophils are still present, their percentage varying between 1 and 20, the percentage of mononuclears is approximately normal, and a few eosinophils and lymphocytes are present. It is, however, questionable whether we may state that the cellular contents of the peritoneal cavity have returned to normal, since neutrophils may persist (from 1 to 10) for from four to six days after the injection, and the eosinophils may remain relatively low for six days or longer. The complete restoration to normal is, therefore, a relatively slow process.

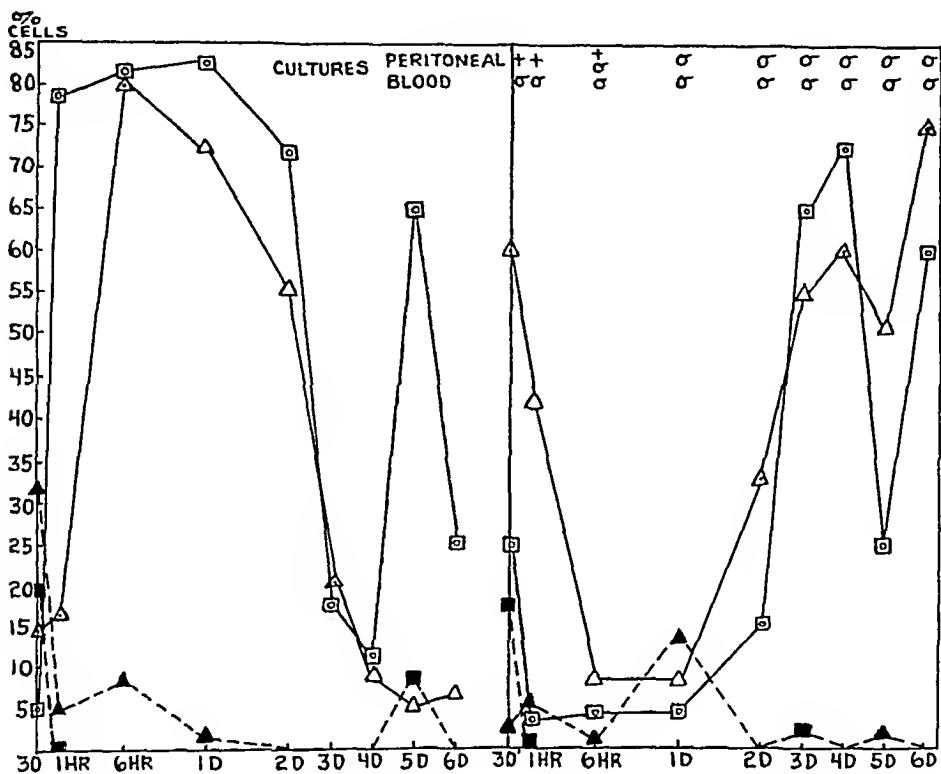


Chart 1—Percentages of neutrophils, mononuclears and phagocytosing cells in peritoneal fluid of animals given intraperitoneal injections of avirulent (rough) pneumococci. Solid lines represent neutrophils (left side of chart) or mononuclears (right side), beaded lines represent phagocytosing cells.

Effect of Nonpathogenic Bacteria—When avirulent or nonpathogenic bacteria were injected (2 cc of a twenty-four hour broth culture of an avirulent strain of hemolytic streptococci, *Corynebacterium pseudodiphtheriae* or avirulent pneumococci) the reaction differed very little from that occurring when a nonliving irritant is injected intraperitoneally. Occasionally the neutrophil percentage was still high at twenty-four hours after injection, and it might remain relatively high (above 25 per cent) at forty-eight hours, in those cases in which the neutrophils remained high, the mononuclear cells rose less rapidly toward the normal percentage. It should be noted that cultures from the peritoneal fluid were positive at thirty minutes, one hour and usually six hours after injection but were never positive at twenty-four hours after injection (chart 1).

Effect of Virulent Bacteria—When virulent pneumococci were injected intraperitoneally there was presented a different picture from that just discussed. It is advisable to separate the animals which succumbed to the infection into groups (1) animals which died in a short period after injection, that is, within the first twenty-four to one hundred and twenty hours, and (2) animals which died after five days. This is principally justified because the reactions tended to be uniform within the earlier period but became more irregular in the later periods. It is not implied that there was an actual uniformity even in the periods through the ninety-sixth hour, the individual animals showed variations, but there was a tendency toward similarity of the reactions.

We may concern ourselves solely with the neutrophils and mononuclears since the eosinophils and lymphocytes were essentially negligible after the sixth hour subsequent to injection of the bacteria. By the sixth hour and occasionally at

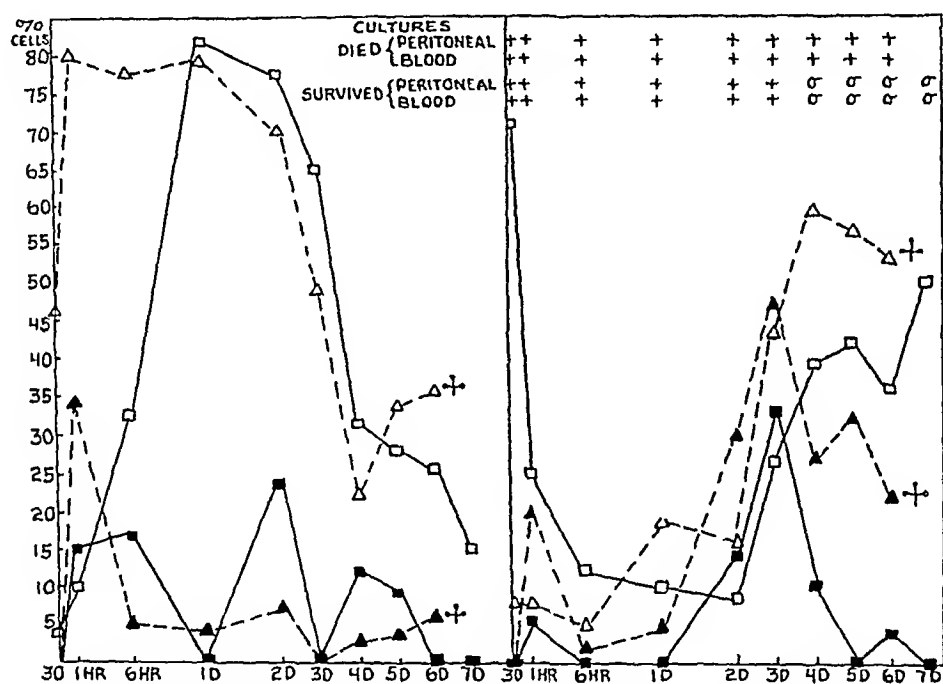


Chart 2—Percentages of neutrophils, mononuclears and phagocytosing cells in peritoneal fluid of animals given intraperitoneal injections of virulent pneumococci. Solid line represents animals that survived, broken line, animals that died. Open figures show neutrophils (left side of chart) or mononuclears (right side), solid figures show phagocytosing cells.

one hour after injection the neutrophils had risen to 90 per cent or higher and continued at this level at the twenty-fourth hour and even on to the forty-eighth hour. Usually, however, by the forty-eighth hour there had occurred a drop in the relative percentage of neutrophils so that these cells might represent from 50 to 75 per cent of the cellular elements. Twenty-four hours later there might be a further fall which, however, rarely brought these cells below 40 per cent of the total, and at ninety-six hours the neutrophils might fall to from 20 to 35 per cent of the total cells. At times, however, there occurred a secondary rise of the neutrophils at the seventy-second or ninety-sixth hour, and even at later periods the neutrophils might remain about 75 per cent of the total cellular elements (chart 2).

The mononuclears fell rapidly and sharply in the first six hours (even at one hour these sometimes fell to from 7 to 25 per cent) and represented only about from 3 to 15 per cent of the cells. At twenty-four hours they might still be present in a low percentage, but often at this time there was evident a slight tendency for them to increase, the percentage at this time was, however, rarely above 25 per cent. After forty-eight hours the percentage might show a further rise to close to 50 as a maximum, however, at this period the mononuclears might still be low, representing only slightly over 10 per cent of the cells. At ninety-six hours their tendency toward an increase was more marked, the mononuclears usually varying between 30 and 65 per cent, and rarely was the percentage under the lower figure at this time.

If we consider those animals which survived for periods longer than one hundred and twenty hours, and in which the peritoneal cultures remained positive throughout the experiment, i. e., until the death of the animal, we find that the percentages of neutrophils and mononuclears were much as in the animals which died before five days, but that after this time considerable variation appeared in the percentages of neutrophils and mononuclears from day to day. It was noted in some animals that after an apparent reduction of the numbers of pneumococci in the peritoneal fluid on some of the earlier days (third and fourth) the neutrophils fell and the mononuclears rose in percentage, but that when the numbers of bacteria increased, the neutrophils rose and the mononuclears fell. Subsequently, however, the neutrophils decreased and the mononuclears increased up to the death of the animals.

Attention may be directed toward the lymphocytic reaction in these more extended infections. The percentage of lymphocytes rose with the early increase in mononuclears and later tended to remain high even while there occurred variations in the percentage of mononuclears. This tendency toward an increase in the lymphocytes was, in general, characteristic of the later stages of infection (at or after the fourth day whether the animal eventually died or survived). There was considerable irregularity in this reaction from one animal to another and even in the same animal from day to day. It is suggested that this increase in lymphocytic percentage is rather a factor of time than a factor associable with development of resistance or progress toward recovery.

Reactions in Animals Recovering Spontaneously—A number of animals which received injections of pneumococci at the same time as those which served for the foregoing observations recovered spontaneously. In all animals considered in this group the peritoneal cultures were positive for the first few days after injection, usually through the fourth or fifth day after injection, but in some cases the positive peritoneal cultures persisted for longer periods. After the cultures were once negative they remained so throughout the period of observation (pneumococci might still be seen within the cells, however, for several additional days). The number of pneumococci in the peritoneal cultures was usually comparable to that in the cultures from animals which died, except possibly on the day before the peritoneal cultures became negative, when the number was at times reduced. In addition, in the cases in which animals recovered spontaneously blood cultures were positive in the earlier days and usually continued positive as long as did the peritoneal cultures (at times the blood cultures were negative twenty-four hours before the peritoneal cultures). It is apparent that in the animals which recovered there had existed a rather severe infection, and that recovery was not dependent on an initial power of resistance existing at the time of injection but must have depended largely on a resistance built up during the period of infection.

We can separate these animals into two groups those in which the infection lasted only from four to five days and those in which the infection lasted longer. The majority belong to the former group, in the first three or four days both the neutrophilic curves and the mononuclear curves were essentially similar to those observed in the animals which eventually succumbed to the infection. At the fourth or fifth day, however, there was practically always a shift in favor of a high percentage of mononuclears and a low percentage of neutrophils in the surviving animals as compared with those which died. It must be remembered that at this period the bacteria either were about to disappear or had disappeared, and conditions were similar to those observed in animals receiving injections either of irritants or of nonpathogenic bacteria at from twenty-four to forty-eight hours after the injection. Except for the fact that the relations of neutrophils and mononuclears were restored toward normal with the disappearance of the bacteria, it was not possible to note any distinct difference in the cellular reactions in animals which died and those which recovered from the infection (chart 2).

Some animals which did not recover until after the fifth day showed positive cultures for from eight to ten days. In these animals there was a rather rapid rise in the percentage of mononuclears in the first few days of the infection (at forty-eight, seventy-two or ninety-six hours). At the same time one noted the relatively early fall in the percentage of neutrophils in the first few days. Beyond this period there occurred rather wide fluctuations in the percentages of neutrophils and mononuclears, this cannot be related to any demonstrable factor associated with the infection. It is possibly of interest to note that immediately before the disappearance of the bacteria (as demonstrated by cultural tests of the peritoneal exudate) the neutrophils seemed to show a definite increase in percentage which might either disappear as the peritoneal culture became negative or persist for a few days thereafter. Naturally, after the peritoneum no longer yielded positive cultures, the neutrophils fell (but only slowly), and in general the mononuclears rose toward their normal percentage. In these animals which eventually recovered one noted irregularities in the percental relations of mononuclears and neutrophils similar to those noted in animals which succumbed to the infection after a long period.

Reactions in Passively Immunized Animals—Experiments were carried out in which guinea-pigs were passively immunized previous to the injection of the usual quantity of virulent pneumococci. Twelve hours before the injection of the bacteria antipneumococcic serum type I was administered subcutaneously (the intra-peritoneal administration was not used so that irritation and resultant cellular reaction in the peritoneal cavity might be avoided). To one series of animals 3 cc of antiserum was administered, three of four animals died. To a second series 3 cc was administered subcutaneously twelve hours before injection, and an additional 3 cc subcutaneously at the time of injection of the bacteria, all of the latter group survived. In the first series blood cultures and peritoneal cultures were positive during the life of the animals, except that in the case of the one which recovered the blood culture was positive at six hours but negative at twenty-four hours, and the peritoneal culture was positive at twenty-four hours but not thereafter. In the experiments in which larger amounts of antiserum were injected, both blood and peritoneal cultures were positive at six hours but negative at twenty-four hours (chart 3).

In spite of these differences in the course of infection the cellular reactions in the first forty-eight hours were essentially similar to those noted in other experiments in which bacteria were injected. The neutrophilic reaction was sustained, and the mononuclear percentage was depressed, tending however to rise. Again

there was noted the difference which appeared when the irritant (the bacteria) disappeared, that is, a sharp increase in the percentage of mononuclears and a fall in that of neutrophils

PHAGOCYTOTIC ACTIVITY IN THE PRESENCE OF AVIRULENT PNEUMOCOCCI (ROUGH)

In four animals given injections of avirulent organisms the pneumococci were not demonstrable in the blood stream at any time, and they usually were absent from the peritoneal cultures at six hours after injection

Thirty minutes after the injection, from 6 to 40 per cent of the neutrophils (present in relatively small numbers) had ingested bacteria. At one hour the

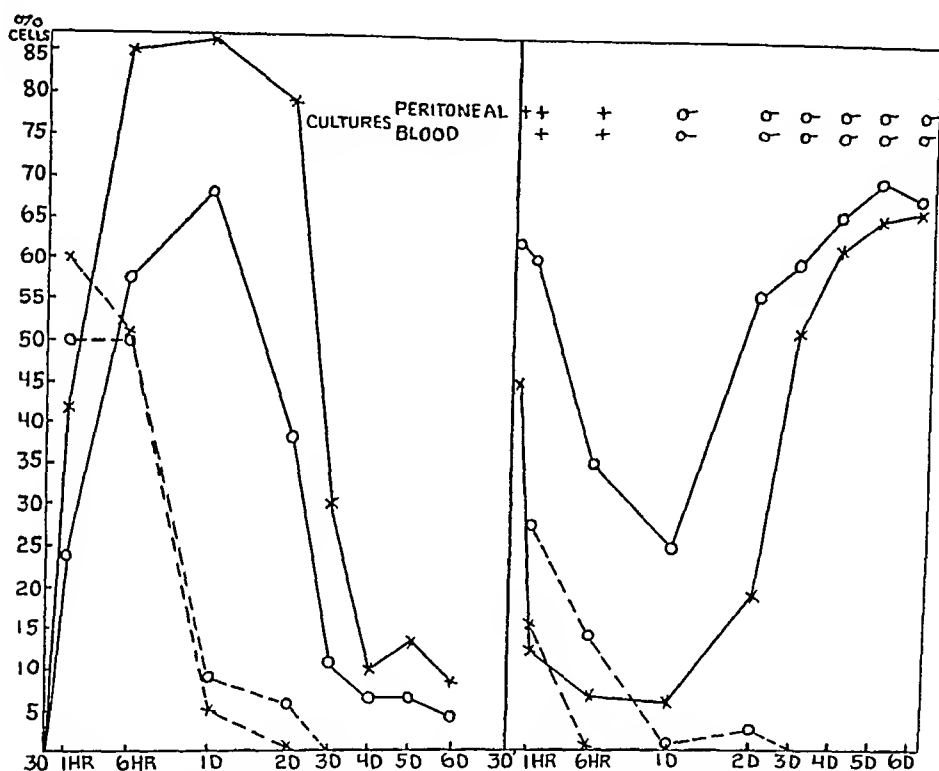


Chart 3—Percentages of neutrophils, mononuclears and phagocytosing cells in peritoneal fluid of passively immunized animals given intraperitoneal injections of virulent pneumococci. Solid lines represent neutrophils (left side of chart) and mononuclears (right side), beaded lines, phagocytosing cells

percentage of actively phagocytic neutrophils had fallen to between 0 and 6, and in most animals the phagocytic activity remained low or could not be observed, in two animals, one examined at four days and the other at five days after infection, bacteria was still seen in 10 and 20 per cent of the neutrophils, respectively

In the earliest period (thirty minutes) the mononuclears showed relatively less phagocytic activity than the neutrophils, in one animal 10 per cent of the mononuclears had taken up bacteria while in the remaining ones from none to 3 per cent of the mononuclears showed phagocytosis. Usually at one and six hours there was no phagocytosis by mononuclears (one animal showed 5 per cent of the cells active at one hour). In most animals no phagocytosis by mononuclears was

evident at or after twenty-four hours, but in one animal 14 per cent were active at twenty-four hours, and in another animal very slight activity (from 2 to 1 per cent) was noted at three days and five days (chart 1)

It is apparent that phagocytic activity is most marked in the very early period after injection of avirulent pneumococci, but that this activity subsides rapidly simultaneously with the disappearance of the bacteria from the cultures

While it has been stated that at thirty minutes the percentage of phagocytes is higher among the neutrophils than among the mononuclears, one should keep in mind the fact that at this time the number of mononuclears is definitely greater than that of the neutrophils, so it is probable that in the early stages the mononuclears are quantitatively actually the more effective in phagocytic activity

Phagocytic Activity in the Presence of Virulent Pneumococci Reactions at from Thirty Minutes to Six or Eight Hours in Animals Which Died—Within the period of the first six to eight hours the phagocytic activity of both neutrophils and mononuclears was quite irregular. In a considerable number of animals, probably about 50 per cent, no phagocytosis could be observed

In a series of fourteen animals at a period thirty minutes after injection phagocytic activity by mononuclears (about 6 per cent) was observed in only one animal, and phagocytosis by neutrophils (about 13 per cent) in one other animal. At one hour, however, phagocytic activity could be noted in about from 20 to 25 per cent of the animals either by neutrophils or by mononuclears or by both. In the case of the neutrophils the percentage of phagocytes rose as high as 50 or was as low as 6, when phagocytosis was evident at this stage there was a tendency for the activity to be fairly marked. At this same period, in some animals, the mononuclears showed phagocytic activity, and usually it was less than that of the neutrophils

At six hours there was a tendency for phagocytic activity to decrease, although in a few animals which had not shown any such activity of the neutrophils at one hour there was evident a marked degree of phagocytosis (in from 40 to 75 per cent), in other animals phagocytic activity was noted in 5 per cent or less of these cells. In the case of the mononuclears the tendency toward little phagocytic activity was more marked than in the neutrophils, and the majority of the animals showed no mononuclear phagocytes, three of the fourteen considered here showed 14, 4 and 4 per cent, respectively

One must, of course, keep in mind (1) the fact that in these early periods the total number of leukocytes was usually small, except possibly at six hours, and (2) that this was a period of changing distribution of the types of cells—the mononuclears were falling and the neutrophils increasing in percentage. Thus if there was noted a high percentage of neutrophilic phagocytes at one hour, this may have represented little actual phagocytic effect, if there was neutrophilic activity at six hours, this may be considered more significant, but the significance would vary in individual cases. Since the mononuclears were decreasing in relation to the neutrophils, mononuclear activity was decreasingly less significant, and the low point of mononuclear phagocytic activity corresponded to the low point of the mononuclear percentage in relation to the total number of cells in the peritoneal fluid

In comparing phagocytic activity in animals which eventually died and in animals which eventually survived it is not possible to note any difference at this stage, one may find relatively active phagocytosis or no phagocytosis in individual animals of each group

Animals Dying Within Forty-Eight Hours—In all of these animals phagocytic activity was marked at twenty-four hours, which was the last period of observation. This activity was evident in both neutrophils and mononuclears. In the case of

the neutrophils, which were, of course, the predominating cells, between 30 and 60 per cent had taken up bacteria. In the case of the mononuclears, which at this stage were relatively few, the percentage of phagocytes varied between 12 and 60. In both types of cells in most of the animals the tendency was toward the higher percentage activity.

Animals Dying Within Seventy-Two Hours—In contrast with the group which died within forty-eight hours after injection, the animals which survived beyond the forty-eight hour period showed lower phagocytic activity at twenty-four hours. In the latter at twenty-four hours between 4 and 35 per cent of the neutrophils showed activity, usually less than 15 per cent, between none and 40 per cent of the mononuclears showed phagocytosis, with a rather large number of animals showing no phagocytes and the majority from no phagocytes to 10 per cent. However, at forty-eight hours these animals without exception showed a marked degree of phagocytic activity in both types of cells. Among the predominant neutrophils the percentage of the phagocytes varied between 65 and 95, and among the mononuclears, between 50 and 95 (with all but one animal showing a percentage above 65).

Both in animals dying before forty-eight hours and in those dying before seventy-two hours large numbers of pneumococcus pairs were found free in the peritoneal fluid, and in most cases the pneumococci practically carpeted the smear. It is evident, then, that the leukocytes had ingested only a small portion of the bacteria, but it was striking that in every case the phagocytic activity had been marked at a period shortly before death of the animals.

Animals Dying Within Six Days—In the animals which survived longer than two days but which died within six days after injection of the bacteria the phagocytic activity within the first six to eight hours was very similar to that previously described and showed the same amount of irregularity. Nothing was observed in this earlier period in relation to the phagocytic reaction which differentiated these animals from those which died before the seventy-two hour period (chart 2).

At twenty-four hours the phagocytosis by neutrophils was usually negligible, often being unobservable, and rarely did more than 5 per cent of the neutrophils show this activity. At forty-eight hours, in the majority of the animals, phagocytic activity was low (still below 5 per cent), although in one animal 33 per cent of the neutrophils had taken up bacteria. From this time on until death the phagocytic action of the neutrophils was as a rule slight, the percentage of phagocytes being between 0 and 5 per cent, but an occasional animal would on one day, rarely on consecutive days, show a percentage as high as 33. On the day before death the neutrophilic phagocytosis was practically uniformly low.

The mononuclears tended to be more active than the neutrophils, but there was rather marked irregularity in different animals. Usually but not always phagocytic activity was evident at twenty-four hours, the percentage of the phagocytes rising as high as 45 in several animals, but in a few animals phagocytosis was absent from these cells or occurred in between 5 and 15 per cent. At forty-eight hours there was usually some phagocytic activity, the percentage of phagocytes in one animal rising as high as 30, but usually it was between 5 and 15, in a few animals at this period no phagocytic activity was observable. From this period onward mononuclear phagocytes were rarely absent, but in some animals the percentage seemed to vary from day to day between 5 and 40, tending possibly to center around 20 to 25. When the mononuclear phagocytosis had been high, a fall usually occurred on the day before death, but even at this time the phagocytic activity of the mononuclears was fairly marked. It should be remembered that in the latter days, third, fourth, etc., the mononuclears were generally tending to increase in

numbers even though with the continued presence of large numbers of leukocytes the neutrophils were probably the dominant cells

Animals Dying After the Sixth Day—In two animals which died after the ninth and before the tenth day certain rather similar tendencies were noted in the mononuclear activity but not in the neutrophilic activity. In one the percentage of neutrophilic phagocytes was increased markedly at the second and third days (from 55 to 60), later continuing at about 2 to 5, but falling to 0 at the eighth and ninth days. In the other animal the neutrophilic phagocytosis was negligible except at one day after injection. In both animals the percentage of mononuclear phagocytes was high (from 70 to 90) in the earlier periods, that is, through the third day. In the next few days it fell more or less gradually to a low of 0 on the seventh day in one animal and to 22 on the eighth day in the other animal. In both animals it rose to between 50 and 70 on the ninth day, which was the last observation before the death of the animals.

Animals Which Survived—In animals which recovered spontaneously phagocytic activity in the early period after infection (from thirty minutes to six or eight hours) by either or both neutrophils and mononuclears was observed at times and was occasionally marked or was at times absent or almost negligible. The phagocytic activity of both neutrophils and mononuclears was essentially similar at the early period in animals which recovered and in those which eventually died.

At twenty-four hours the percentage of neutrophils that had ingested pneumococci was usually low, being 0 or rarely above 5, but that of mononuclears showing this activity was usually high (between 15 and 90), although such activity was absent in some animals. In general, the phagocytic activity in the group which survived corresponded to that which had been noted in guinea-pigs eventually dying after surviving beyond two days (chart 2).

At forty-eight hours there was often a tendency for the neutrophilic phagocytosis to be more marked than at twenty-four hours, but a number of the animals showed little phagocytosis at this period. Occasionally the percentage of neutrophilic phagocytes rose to 25, but usually it was below 5. The phagocytic activity of the mononuclears might now show a diminution when it had previously been high, the percentage of phagocytes falling to 5 or less, when it had been absent, phagocytic activity did at times rise, the percentage of phagocytes reaching a maximum between 25 and 40. Again in a few animals no phagocytosis by mononuclears was observed.

After forty-eight hours there occurred at times an increase of neutrophilic phagocytosis, which might be brief and variable or might extend over several days. In a number of animals some phagocytic activity of the neutrophils was noted at three days or over a period from the third through the sixth day, after which time, however, pneumococci were rarely seen in the neutrophils. In general, the mononuclears tended to show less phagocytic activity after forty-eight hours, and there occurred a more or less uniform tendency toward a decrease extending through the fifth, sixth and seventh day. In individual animals there were, however, irregularities in this descending curve, variations occurring from day to day. Usually in those cases in which the peritoneal culture was negative before or at the sixth day little or no phagocytic activity of either neutrophils or mononuclears could be noted at later periods, occasionally an isolated cell containing a few bacteria was found, but it might probably be considered as a cell which had failed to digest the bacteria taken up at an earlier period.

At later stages, from ten to fifteen days after injection, occasionally there appeared showers of mononuclears which contained numbers of bacteria, but these

appearances were usually brief in duration and were not accompanied by an increase in the number of neutrophils or any evidence of neutrophilic phagocytic activity.

In considering the phagocytic activity appearing in the animals which recovered spontaneously from the infection it was essential to bear in mind the fact that in these animals at a certain stage the peritoneal cultures became negative, in contrast to the fact that in the animals that died the cultures generally remained positive throughout. In a majority of the cases in which animals recovered, the cultures from the peritoneal fluid were negative after the sixth or seventh day, in a number of cases the cultures were negative after the third or fourth day. It should also be noted that during the first few days after the injection the blood cultures were also positive. It is apparent that with the disappearance of the pneumococci the phagocytic activity should fall. However, evidence of phagocytosis persisted for a few days even after the cultures of the peritoneal fluid were negative. This disappearance of bacteria from the peritoneal fluid must be given consideration when we compare the phagocytic activity in animals which survived with that occurring in animals which died.

Immune Animals—In a group receiving only one injection of antiserum the blood cultures were positive at thirty minutes after infection, and in those which died both the peritoneal and the blood cultures were positive until death. In those animals which had received inadequate protection, the phagocytic activity at thirty minutes was negligible or absent as concerned both the neutrophils and the mononuclears, except in one animal (which did not survive). At one hour after injection of the bacteria there was in some cases slight phagocytic activity of the neutrophils, but this was absent in one half of the animals, the mononuclears in three of the four animals showed no phagocytosis. At six hours neutrophilic phagocytosis was more or less marked (between 5 and 25 per cent of the cells showing it), but mononuclear phagocytosis was absent in all animals. At twenty-four hours practically all animals revealed fairly active phagocytic activity (except one animal which showed no phagocytic activity of the mononuclears). From this time on until the death of the animals phagocytosis was more or less marked in all, and the usual high phagocytic activity was noted in the mononuclears at the last observation before death. In the case of the guinea-pig that survived the cultures of the peritoneal fluid were negative after twenty-four hours (the blood culture at twenty-four hours was negative), and the phagocytic activity of both types of cells fell sharply after twenty-four hours, although bacteria were seen in the cells (both neutrophils and mononuclears) even through the fifth day.

In the animals which were apparently adequately protected both the peritoneal and the blood cultures were positive at one hour but were negative after six hours and remained negative.

The phagocytic activity of both neutrophils and mononuclears was evident almost entirely within the first few hours. At one hour after infection between 50 and 60 per cent of the neutrophils (in one guinea-pig, however, less than 5 per cent) showed phagocytic activity, and between 5 and 30 per cent of the mononuclears showed this activity (the lowest percentage appearing in the same animal which showed only a small percentage of neutrophilic phagocytes). At six hours both the neutrophilic and the mononuclear percentage of phagocytes had fallen, the former to from 30 to 50 (in the inactive animal, to 0) and the mononuclear to 0 in three of the four animals and to about 4 in the fourth. At twenty-four hours no mononuclears showed activity, and the percentage of polymorphonuclears that did was between 8 and 0. After twenty-four hours the phagocytic activity had practically ceased, but a few cells, both mononuclear and polymorphonuclear, contained bacteria two days after injection (chart 3).

It is of interest to note the rather striking absence of phagocytosis in one animal of this series. Only at one hour after injection was slight phagocytosis noted (in about 5 per cent of both mononuclears and polymorphonuclears), and thereafter no phagocytic activity was noted. The cultures from this animal were identical with those from the remainder of the group.

COMMENT

It may be questioned whether this study of the cellular and phagocytic reactions during the course of infection by means of smears of the peritoneal fluid serves to give an adequate picture of the processes occurring in the body of the invaded host. Since the pneumococcic infection becomes a general infection shortly after the injection of the bacteria it is recognized that cellular and phagocytic processes must take place in other parts of the body. However, it may be assumed that this study gives a partial picture of the processes in the infected animal.

It is also to be noted as an essential limitation in these studies that the total number of cells in the peritoneal fluid during the course of the infection was not determined, it was impossible to determine this without killing the animals and thus defeating the possibility of determining the outcome of the infection.

It is also recognized that in the peritoneal cavity the omentum plays an important rôle in the course of infection.² A few animals were killed and smears from the omentum made at the early periods of infection with the idea of permitting a comparison between these smears and those of the peritoneal fluid. The changes in the distribution of cells at thirty minutes, one hour and six hours in the omental smears were essentially similar to those which had been noted in the free cells of the peritoneal fluid at the same periods. That is, the mononuclears decreased, the neutrophils increased, and the eosinophils and lymphocytes both diminished in numbers. As regards phagocytosis, at thirty minutes the phagocytic activity of the neutrophils and mononuclears was high, being observed in about 75 per cent of the mononuclears and 40 per cent of the neutrophils, at one hour the percentage of the mononuclears showing this activity had fallen markedly to about 55 and that of the neutrophils had risen to about 50. However, at six hours the percentage of neutrophilic phagocytes was between 15 and 20 per cent and that of mononuclear phagocytes was about 3. It seems apparent that the phagocytic activity of the wandering cells associated with the omentum is very much like that of the cells free in the peritoneal fluid. There is, then, justification for assuming that the study of the peri-

² Durham, H. E. *J. Path. & Bact.* **4**: 338, 1897. Dudgeon, L. S., and Ross, A. *Tr. Path. Soc., London* **57**: 155, 1906. Burton, B. H., and Torrey, J. C. *J. M. Research* **15**: 55 and 73, 1906.

toneal fluid gives a fair indication of what is occurring in the peritoneal cavity as a whole

Through all these studies of the cellular response occurring in the peritoneal cavity subsequent to injection of irritant substances of various types one notes in the early stages the same sort of reaction. In large part, the response up through the first twenty-four hours is the same. An increase in the number of neutrophils begins to become evident at one hour after the injection and reaches a maximum sometimes at six hours and usually by twenty-four hours, a relative decrease appears in the mononuclears which is possibly actual in the first few hours, in addition, the lymphocytes tend to diminish in number or disappear, the eosinophils show a relative increase in the first few hours (probably not, however, an actual increase), but soon their number is negligible. This may be considered the routine response to introduction of an irritant. It is not assumed that in these first few hours the change in the cellular picture in the peritoneal cavity is principally an active one, but rather that the decrease in mononuclears is due to the adherence of these cells to the omentum.²

After this first twenty-four hour period the reaction depends on the persistence of the irritant. When chemical substances or nonpathogenic bacteria have been injected, the entire reaction tends to subside rapidly. The percentage of neutrophils falls, and that of mononuclears rises so that by forty-eight or seventy-two hours cells of the latter type dominate the picture, in addition, there may be noted in many cases a more or less marked increase of the lymphocytes. The eosinophils do not tend to return to the normal percentage for seven days or even longer, and neutrophils, which are either few or absent in normal peritoneal fluid, tend to persist for an equally long period.

If, however, the irritant, in the form of bacteria, persists in the peritoneal cavity, there is not evident the same tendency toward restoration of the normal relations of the cellular elements. The neutrophils tend to remain present in relatively large quantities for several days. Even though the infection persists, however, there may be noted the tendency for the relative number of neutrophils to decrease and for that of mononuclears to increase. There may occur irregular fluctuations in the relations of the two types of cells.

The tendency for the mononuclears to increase at or after the twenty-fourth hour is observed in all animals which survive for forty-eight hours. This increase occurs even though the infecting agent is present. It is possible that the increase in mononuclears is related principally to time and is not dependent on the stimulus (irritant), since in animals receiving injections of avirulent organisms the rise of mononuclears is also noted after a somewhat similar lapse of time. However, in the animals receiving injections of avirulent organisms the

increase in mononuclears is apparent earlier than in those receiving injections of virulent organisms, this difference may be apparent rather than real and may depend on the neutrophilic reaction resulting from the presence of virulent organisms masking the mononuclear increase. The assumption may be made that the neutrophilic reaction is essentially dependent on the irritant effect of the bacteria and their products, while in contrast the mononuclear reaction is largely dependent on time and in part independent of the irritant.

If one compares the cellular reactions in animals which succumbed to the infection with that in animals which recovered, there is practically no difference to be noted except, of course, in the recovering animals at the stage when the bacteria disappear from the peritoneal cavity. When the pneumococci have disappeared, the neutrophils tend to fall rapidly and the mononuclears to increase, it is certainly justifiable from the study of the cellular reactions in individual animals to assume that the direction which the reaction takes at this period is the result of the disappearance of the irritant rather than to assume that the pneumococci disappear as a result of the changes in the cellular elements.

If this interpretation of the cellular reaction is correct, there is nothing in the quantitative characters of the cellular reaction which can be related to either recovery or fatal termination. The cellular reaction in the peritoneal cavity cannot, then, be related to the processes of resistance to infection but must be assumed to be essentially determined by the factors of irritation or stimulation as exerted by the pneumococci.

If one compares the phagocytic activity noted in normal animals which succumb to the infection with the activity in normal animals which survive one finds very little difference between the two lots.

In the earlier periods through six or eight hours the same tendency toward fairly active phagocytosis may be noted in a considerable number of animals, irregular it is true, varying as to neutrophils or mononuclears, but definite in many animals. In a number of cases in this study both in animals which survived and in those which succumbed, no phagocytic activity by either type of cell was noted. It may probably be considered that in this early period before twenty-four hours after infection the phagocytic reactions in normal animals given injections of virulent bacteria are essentially nonspecific and not related to the possibility of death or recovery.

While usually both in animals which survived the infection and in those which died some time after the third day, phagocytic activity was depressed or absent at twenty-four hours or even occasionally at forty-eight hours, in those animals which died before the second or third day phagocytosis was usually marked at the last observation previous to the death of the guinea-pig. It may be suggested that the active phagocy-

tosis was due to the presence of large numbers of bacteria, but in view of the fact that enormous numbers were present on the slides of the peritoneal fluid of all animals at this stage it is not likely that this factor played a rôle

At twenty-four hours the phagocytic activity of the neutrophils was, with the exceptions mentioned, usually at a low ebb both in animals which survived and in those which eventually died, at this time phagocytic activity of the mononuclears was usually evident, at times even marked, but occasionally absent

After the twenty-four hour period the neutrophilic activity was usually absent, although an occasional guinea-pig showed phagocytic activity of this cell at the second, third or fourth day, this occurred in animals regardless of the later course of infection. In both groups of animals the mononuclear phagocytosis was quite marked on the second day and through the latter periods, usually tending to show a slight increase at the observation just preceding the death of the animal. The mononuclear phagocytosis in the later periods is all the more significant since the percentage of these cells in the peritoneal fluid was usually rising in the later periods

When one compares the phagocytosis in animals which recover with that in animals which die, one must keep in mind the fact that in the former at a certain stage the bacteria are no longer demonstrable by culture in the peritoneal fluid. In discussing cellular reactions it has been stated that the effect of removal of the irritating agent should become evident in the cellular reaction, and in a similar fashion it is apparent that the disappearance of the bacteria should affect the phagocytic activity. If one compares the phagocytic activity in the animals which recover with that in the animals which die one finds up to the time of disappearance of the pneumococcus similar tendencies and similar variations. After the peritoneal cultures have become negative in animals which survive, the phagocytosis naturally falls

If one considers the phagocytosis which occurs in the later stages of infection in practically all animals and especially that at the period shortly preceding death in animals which succumb to the infection it is difficult to determine whether the leukocytes are ingesting only bacteria which have lost their virulence or are ingesting bacteria regardless of virulence. Cultures of the bacteria obtained from the peritoneal fluid have not shown any rough colonies or any diminution of virulence for mice. Certainly, one would have to assume that at periods twenty-four hours or later after infection there should be present only virulent organisms, since by selection and adaptation the avirulent organisms should have been lost. The evidence presented suggests that *in vivo*, in the course of infection, the wandering cells of the host may and do ingest bacteria which are virulent

The phagocytic activity of the eosinophils should be noted. In the early stages of the infection these cells are usually active, in many animals nearly 100 per cent of the eosinophils may contain bacteria. Usually these cells take up few bacteria, only one or two pairs of pneumococci are usually found within the eosinophil. This early phagocytic activity may be noted in animals which survive and in those which die, and is independent of the early neutrophilic or mononuclear phagocytosis. Like the general trend of phagocytosis the eosinophilic phagocytosis is not noted in some animals.

In the early stages of infection, before twenty-four hours, there is a tendency for both neutrophils and mononuclears to take up bacteria. With due allowance for the differences noted in individual lots and individual animals, it seems that at this stage, when the preexistent mononuclears are present and the neutrophils are beginning to pass out from the vascular system into the peritoneal fluid, both these types of cells possess phagocytic ability. After twenty-four hours there occurs a period in which nearly always the mononuclear cells show a considerable degree of phagocytosis, and the neutrophils only occasionally show it. In animals which die before forty-eight hours or seventy-two hours, however, the neutrophils show marked activity, in others surviving longer periods the neutrophilic action is only rarely noted. At periods beyond forty-eight hours the phagocytic activity in animals which die and in those which survive is chiefly confined to the mononuclears. This is, of course, not due to either absence or small numbers of neutrophils. It is evident that in the later stages of infection the neutrophils play but little rôle in phagocytic activity, and the mononuclears take over this function. It appears that although the phagocytic activity of neutrophils and mononuclears may be essentially equivalent when tested *in vitro*,³ this is not the case in the course of infection, and at the later stages of infection the mononuclears are the active phagocytic cells even though neutrophils may be present in the peritoneal exudate in large numbers. In this connection one may recall the stress which Gay and his co-workers⁴ laid on the clasmatoocytes in resistance to streptococcic infection.

If our results represent a fair cross-section of the phagocytic activity in fatal infection and in infection with recovery, it cannot be stated that phagocytic activity as shown by the free cells of the peritoneal cavity is a factor in successful resistance developing during the course of infection.

If one compares the phagocytic activity in satisfactorily immunized animals with that appearing in normal ones (either dying or surviving),

3 Lucke, B., Strumia, M., Mudd, S., McCutcheon, M., and Mudd, E. B. H. *J. Immunol.* **24**: 455, 1933.

4 Gay, F. P., Clark, A. R., and Linton, R. W. *Arch. Path.* **1**: 857, 1926.

it is apparent that the reactions are rather different. In animals which are completely immunized practically all phagocytic activity occurs within the first six to twenty-four hours, and after this period few phagocytic cells are noted. To a certain extent this corresponds with the phagocytic activity noted when avirulent or nonpathogenic bacteria are injected into normal animals, but is more rapid and more complete. It is what one might expect as a result of sensitization of the bacteria through the presence of antibodies. It is possible that these differences in phagocytic activity between immunized and normal animals denote differences in the mechanisms leading to recovery, so that one cannot assume that the mechanism of resistance in the immune animal and that of resistance in the spontaneously recovered animal are identical. These differences may lie in the factors concerned in the two processes or may lie in the time and sequence of the various activities within the host, but the differences observed are too striking to pass over with simply the assumption that the activity of antibodies aiding in phagocytosis accounts for the apparent differences.

Since there is the suggestion that neither the cellular reaction nor the phagocytic activity can serve to explain the phenomenon of spontaneous recovery from infection, we have to seek for some other factor as the vital one concerned. Naturally, we turn to the concept of antibody activity, and this phase is now under investigation. It is interesting to note, however, that we did not observe in recovering animals the increase of phagocytic activity with the approach of recovery which may be expected if the tropic activity of antibodies is the vital one concerned. Furthermore, in a few animals which were apparently highly resistant to pneumococcal infection (animals which had been rendered immune with antiserum or which had recovered from infection and later were again infected with pneumococci) the peritoneal fluid, after injection of the bacteria, remained sterile even at thirty minutes, and no phagocytosis and very little cellular reaction were noted in this fluid at any time. Thus in the highly immunized animal evident reactions, at least as concerns the free cells in the peritoneal cavity, were absent. Either the mechanism of resistance is associated with immediate engulfment of the bacteria and fixation of the bacteria on the walls of the peritoneal cavity (the omentum), or cellular reactions are negligible factors in the resistance. It may be noted that the reactions related to clearing up of the infection in these animals were more striking and proceeded more rapidly than did the reactions in normal animals given intraperitoneal injections of nonpathogenic bacteria, again suggesting that the mechanisms operating in natural immunity, in active immunity and in spontaneous recovery may be differentiated either in toto or in the temporal sequence of the factors concerned.

In support of the concept that phagocytic activity may be of secondary importance is the observation of Mackie, van Rooyen and Finkelstein⁵ that, in the presence of serum, leukocytes possess a bactericidal activity separable from their phagocytic activity

It is of interest, finally, to note that in the animals which recovered spontaneously the time of recovery as measured by the disappearance of pneumococci from the cultures of the peritoneal fluid varied between the third and seventh day after infection. Excluding one case in which the cultures were negative at the second day and one in which the cultures were not negative until the tenth day, we find four cases in which the cultures became negative on the third, three on the fourth, four on the fifth and one on the sixth day after infection. It seems that this tendency for the crisis (if it may be so called) to appear before the seventh day of infection is of some significance and must be given consideration in further studies

SUMMARY

In studying the cellular reactions in the peritoneal cavity of the guinea-pig subsequent to intraperitoneal infection with *Pneumococcus* type I it was not possible to note any differences between animals which died and animals which recovered spontaneously or any differences in the cellular reactions which served to explain the mechanism of recovery

It has not been possible to observe in the wandering cells of the peritoneal fluid any difference in phagocytic activity of neutrophils or of mononuclears as between animals that survived spontaneously and those that died. This applies to the amount of phagocytosis and to the sequence of the activity

The question is therefore raised as to whether phagocytic activity plays a rôle in spontaneously arising resistance to infection

The results suggest that the process of resistance in immunized animals and that in animals spontaneously recovering from infection are not identical. This may be due to a difference in the factors concerned or simply to differences in the intervals of time and the sequences

It does not appear that the phagocytic activity of neutrophils and that of mononuclears are identical in the various stages of infection. The neutrophils appear to lose phagocytic activity in later stages while the mononuclears retain their activity

⁵ Mackie, T. F., van Rooyen, C. E., and Finkelstein, M. H. *J. Path. & Bact.* **39** 89, 1934

Case Reports

DWARFISM ASSOCIATED WITH LINGUAL GOITER AND CYSTIC HYPOPHYSIS

H GIDEON WELLS, M D, CHICAGO

As far as I have been able to learn from a search of the literature on dwarfism, the following case is unique in some respects, and hence it is placed on record

REPORT OF A CASE

A woman, aged 72, was examined post mortem at the Oak Forest Infirmary on Oct 25, 1933, four days after her death in this institution, the poor-farm of Chicago and Cook County, where she had lived for twenty-three years. It was impossible to find any members of her family or any one who knew anything about her early life. Her name indicated that she was probably of Irish descent. According to the records she was admitted to the Oak Forest Infirmary in 1910 because of feeble-mindedness, she came from the Chicago State Hospital, where she had been since 1896, at which time her age was given as 35. She was quiet and able to act as doorkeeper in the ward for mentally defective women. No history was obtainable. A roentgen examination could not be made because of the patient's obstinate attitude. It was reported that she was in a failing condition because of weakness without special symptoms in April 1933, but she was not bedfast until August 1933. She died on Oct 21, 1933, there were no clinical records of significance during her period of stay at the Oak Forest Infirmary.

No records were available as to her previous history. She never spoke unless spoken to, and her replies were difficult to understand (possibly because of the lingual goiter). She was decidedly substandard mentally, she did not read or write, and although she was able to dress and undress herself and to take care of her physical needs she did not change to clean clothes unless she was told to. Nevertheless she was able to act as doorkeeper in the irresponsible women's ward, and she guarded the door jealously, never permitting patients to escape but admitting the proper attendants. She could not be made to retire until the physician had made the night rounds. When crossed she showed a violent temper and scolded vigorously but unintelligibly. No persons had visited her in many years, and nothing could be learned about her family and relatives. There were no records at the Chicago State Hospital. Dr Ralph Hamill wrote as follows concerning her behavior while in that institution: "I remember a dwarf that I think is the one you refer to, although I should be surprised if that woman had lived as long as this. The patient I refer to was in the poorhouse. She was used as a messenger by some of the nurses and could be counted on to deliver messages. However, she chuckled much to herself, passed remarks on the side that were not a part of the message and obviously lived some kind of an imaginary life quite independent of the reality. There are no records that I know of, and I know nothing of her family or early history."

From the Department of Pathology, University of Chicago, and the Otho S A Sprague Memorial Institute

It is to be regretted that no study of her basal metabolism was ever made, but as she lived as an abandoned mentally defective person on a county poor-farm she was not made the subject of a clinical study, and she outlived her association with those who knew her early history

Postmortem Examination—The body was that of an extremely emaciated white female dwarf, somewhat short-limbed, appearing to be at least 80 years of age. Practically she was nothing but skin and bones. The typical face of the achondroplastic dwarf was lacking (fig 1), as the nose was retrousse but fairly sharp and had a drawn appearance because of the prominence of the inner end of each malar bone. The distance between the eyes was somewhat increased because the glabella was disproportionately broad. The skull was approximately symmetrical, but the



Fig 1—Profile of dwarf showing absence of cretin characteristics

left eyebrow was 0.5 cm higher than the right. The skull was not rachitic. The posterior portion of the skull cap was somewhat flattened, and the skull was of less than normal size.

The external measurements of the body were taken. The total length, standing, was 124.5 cm (49 inches), and sitting, 72 cm (28 $\frac{3}{8}$ inches), the total weight eviscerated, 29 $\frac{1}{4}$ pounds (13.33 Kg), the calculated weight (total), from 39 to 40 pounds (17.7 to 18.1 Kg), the length of the body (from the upper edge of the manubrium to the lower edge of the symphysis), 46 cm, the total length of the upper extremity, 50.5 cm, and the transverse width of the shoulders, 26 cm. The lengths of various bones were as follows: humerus (from the top of the head to the lower border of the external epicondyle), 21.8 cm, radius (from the tip of the styloid process to the top of the head), 16 cm, ulna (from the tip of the

styloid to the tip of the olecranon process), 18.4 cm, carpus, 2.7 cm, third metacarpal, 4.3 cm, third proximal phalanx, 4.5 cm, third middle phalanx, 2.8 cm, third distal phalanx, 1.8 cm (a close approximation). The total length of the lower extremity (from the anterior superior spine to the styloid) was 57.2 cm, that of the femur (from the upper extremity of the greater trochanter to the outer margin of the surface of the joint), 29 cm, the length of the tibia from the surface of the joint to the styloid process measured 23.5 cm, the total length of the fibula was 24 cm. The patella measured 4.7 by 4.2 cm. The total length of the foot was 18.5 cm. The measurements of the pelvis were as follows: intercrustous, 25.5 cm, interspinous, 23.5 cm, baudelocque, 18 cm, and bitrochanteric, 27.5 cm. The measurements of the head were as follows: biparietal, 13.5 cm, bitemporal, 11.6 cm, fronto-occipital, 16.6 cm, occipitontal, 23.5 cm, maximum anteroposterior, 20 cm, maximum vertical, 20 cm, circumference, 53 cm.

The hands were small in proportion to the feet and were not of the trident type. The fingers were slender and graceful, not clubbed. The teeth were missing, and the alveolar processes were absorbed. The superficial lymph nodes and the thyroid were not palpable. The mammary glands were very small but apparently had been slightly developed, the areolae were devoid of pigmentation. The skin was extremely atrophic (apparently more so than is usual in senile persons), there was no growth of hair on the axillae or on the body, with the exception of a few hairs in the pubic region. The joints of the upper extremities all moved freely, but the hips were restricted in adduction by fibrous bands which could be stretched readily. The knee joints were in a fixed semiflexed position but could be extended under pressure. The pelvis was relatively roomy, the distance between the anterior superior iliac spines being 24 cm. The external genitalia were normally formed but were hypoplastic. There were protruding hemorrhoids. There were decubitus ulcers over the sacrum, both great trochanters and the left fifth metatarsal bone.

The large serous cavities showed no significant changes. The immediate cause of death was apparently asphyxia, as the larynx, trachea and main bronchi were all completely plugged by a solid mass of granular material, apparently regurgitated cereal. The smaller bronchioles were filled with pus, and there was bronchopneumonia in the posterior half of the right lung.

Internal Organs Most of the viscera showed no changes except those associated with the dwarfism and with senility. The heart weighed 180 Gm, in the lower part of the interventricular septum it showed extensive fibrosis due to atherosclerosis of the coronary arteries. The aorta showed moderate senile atherosclerosis. The liver weighed 560 Gm and showed merely small hepatic cells and slight fatty changes. The spleen weighed only 30 Gm, it contained an abundance of lymphoid tissue, the reduction in size was apparently due to loss of the pulp. The pancreas weighed 60 Gm and showed no changes. The cortex of the suprarenal glands was poor in lipoids, the medulla was normal. Both suprarenal glands were small and of normal shape, and the right gland measured 30 by 16 by 4 mm, the left, 37 by 13 by 4 mm, each weighed approximately 2 Gm. As seen microscopically the cortex was unusually poor in lipid, but the cellular structure and arrangement seemed entirely normal. The medullary cells contained an abundance of brown pigment. The capsular arteries were not excessively sclerotic. A few rather large accumulations of round cells were present in the medulla. The kidneys together weighed 140 Gm and appeared to be of nearly normal appearance except for occasional small cysts, and although the medium-sized arteries were much thickened the arterioles were not affected, and there was very little evidence of arteriosclerotic scarring. The cortex seemed almost normal. The

ureters and urinary bladder were normal. No abnormalities were found in the alimentary canal. The lymph nodes in general were normal for the age, one peribronchial node being calcified.

Reproductive Organs The generative organs seemed to have reached an early stage of complete maturity but had apparently retrogressed or ceased to develop shortly after early puberty, for there were a few pubic hairs, and a microscopic examination of the ovaries showed occasional corpora albicantia, indicating that there had been some ovulation, but most of the ovary was devoid of such scars and consisted of homogeneous ovarian stroma. There was one small follicular cyst. Grossly the uterus was of distinctly infantile character, the corpus measured 3 cm and the cervix also was 3 cm long and formed more of the entire uterine mass than the corpus, the entire uterus being 6 by 4 cm. The opening of the cervix was of normal size, and its canal was equal in length and diameter to that of the uterus. The cervical canal showed vaginal markings. The whole organ appeared small. The ovaries were extremely small and of equal size. They measured 1.5 by 0.7 by 0.5 cm. There was no gross evidence of corpora albicantia. The vagina was normal except for a few small round pigmented spots. The hymen was intact.

The whole of the external genitalia seemed to share in the extreme emaciation of the body, and the perineum and perineal structures were tightly stretched over the bony pelvis. The adipose tissue of the mons had almost totally disappeared, as had the adipose tissue of the labia majora. The skin of the region shared in the general atrophy of the skin and subcutaneous tissues that was so marked everywhere else over the body. There were a very few fine brown hairs over the mons veneris and a few more along the outer side of the labia majora extending backward as far as the anus. The labia majora were atrophic and covered with shiny, thin, atrophic skin, although the mucocutaneous junction was recognizable. They were well developed anteriorly. There was no posterior fourchet, and the labia were lost in the perineum while widely separated. The perineum was 4 cm long, flattened and covered with thin, shiny, atrophic skin. The labia minora were small but well developed, 1.5 cm long and 0.9 cm wide. They united in a normal manner anteriorly to form the frenum of the clitoris and blended with the labia majora posteriorly opposite the middle of the vaginal opening. The glans clitoris was very small and was barely recognizable since it was covered with a large, well developed, unusually thick but apparently normally formed prepuce. The vaginal opening, gaping and distorted by postmortem examination, measured 2.2 cm in its greatest diameter.

Macroscopic Examination of the Structures of the Neck Of particular interest was the condition of the thyroid, which seemed to exist only in the form of a lingual goiter. The tongue had a flat, atrophic mucosa. Immediately below and to the left of the foramen caecum was a globular submucous swelling, approximately spherical and 2.7 cm in diameter. When incised this mass contained an encapsulated brownish nodule of soft tissue about 1.5 cm in diameter, not presenting the appearance of thyroid tissue and showing no visible colloid or cysts. The thyroid gland was absent from the normal position. When the lingual mass was opened from beneath it was found to be disk-shaped, measuring 2.5 by 2.3 cm by 1 cm. It could be freed from its dense fibrous capsule with relative ease by blunt dissection, excepting over its dome, where it was attached to the lingual mucosa by tissue as hard as cartilage. Its cut surfaces showed an irregular mixture of deep yellow-brown glandular tissue with irregular masses of firm glistening grayish-white tissue. There was no recognizable colloid. Over the dome of the mass the pale fibrous type of tissue was preponderant. Elsewhere the fibrous

areas were scattered irregularly throughout the center of the mass, and the periphery contained brown glandular tissue. There was no similar-appearing tissue either along the midline anterior to the thyroid cartilage or at the usual site of the lateral lobes.

The dissection of the organs of the neck by Dr. Eleanor Humphreys disclosed that on either side of the trachea were two peculiar small bodies (fig. 2) well encapsulated and easily demarcated from the adjacent tissue. The larger, on the right, was an L-shaped body. The pointed upper end of the long arm of the L reached almost to the base of the first tracheal cartilage and was 1.5 cm long. The blunt, rounded short arm, pointing laterally, was 1 cm long. Below the pointed upper end the width of the arm varied from 3 to 5 mm, and the thickness averaged 3 mm. The upper end was composed of solid pale tissue. Below this was a part in which a translucent cyst, about 3 by 2 by 2 mm, occupied one side of the long arm, crowding some brownish firm tissue to the side adjoining the trachea. The rest of the mass was a flabby, brownish, finely lobulated structure, resembling atrophic fat.

The mass on the other side was similar in general features, but it was smaller and more V-shaped. Both arms were relatively short and thick, and the tip of the lower was occupied by a cyst. When this was cut, a small solid, free body like a rice grain escaped. This was about 2 by 2 by 3 mm. Each arm measured about 1 cm in length by 3 by 3 mm. This mass lay at a slightly lower level than that on the other side.

A search of the tissues on both sides of the trachea did not reveal any discrete pale bodies resembling the parathyroid glands.

Microscopic Examination of the Tissues of the Neck. The microscopic examination of the tissues of the neck showed no substance resembling thyroid tissue, the presence of such tissue was limited to the lingual mass. This mass consisted mostly of necrotic fibrous tissue in which nuclei were scanty and evidences of previous glandular structure difficult to detect. Blood pigment was seen in many areas. Only at the periphery were traces of recognizable thyroid tissue to be found, this consisting of a thin zone of atrophic acini, from one to six acini deep, sometimes empty and sometimes containing a small accumulation of colloid. The epithelial cells lining these acini were scanty, they were entirely absent in many of the acini, and when present they were generally very small, exhibiting as a rule little more than a small pyknotic nucleus and no recognizable cytoplasm. There were also acini that were indicated chiefly by an outline with little recognizable structure. The general appearance resembled that often seen in old fibrosed adenomatous nodules in which frequent hemorrhage and necrosis have occurred until the adenoma has been nearly completely destroyed. However, there was no marked calcification or ossification such as is often seen in such fibrosed adenomas.

Two normal, well developed parathyroid glands were present, one on each side of the larynx (R2 and L1, fig. 2). In the vicinity of each parathyroid gland were small cysts lined with a few layers of flattened epithelium which was not keratinized. No more complex epithelial structures were found, nor anything resembling thymus tissue, possibly the epithelial structures represented undeveloped embryonic anlage of the thymus. There was also some adipose tissue, which was of the adult type and not like "brown fat."

The carotid gland was found on the left side by means of serial sections made through the vicinity of the bifurcation of the carotid. It seemed to be small and diffuse but otherwise normal. The right gland was not sought.

The pineal gland appeared to be normal for the patient's age, it contained numerous calcific bodies.

No thymus tissue could be detected in the usual site.

ADDITIONAL EXAMINATION

Structures of the Neck—Dr George M. Curtis, who examined the tissues removed from the neck, thought that the nodule found medial to the bifurcation of the right carotid showed a resemblance to thymus tissue. The lingual thyroid, he stated, "is characteristic of the rudimentary thyroid gland found in the typical dwarf cretin."

As to the other structures in the neck designated in figure 2, Dr Curtis reported the following observations:

Right L-Shaped Body (fig 2) Section R1 (proximal end) revealed alveoli and several cysts with a lining of stratified squamous epithelium. The picture was compatible with the early embryonic thyroid gland. On the other hand, since the thyroid was mainly lingual in its origin and had not descended, I should regard this tissue as having originated from one of the brachial epithelial bodies.

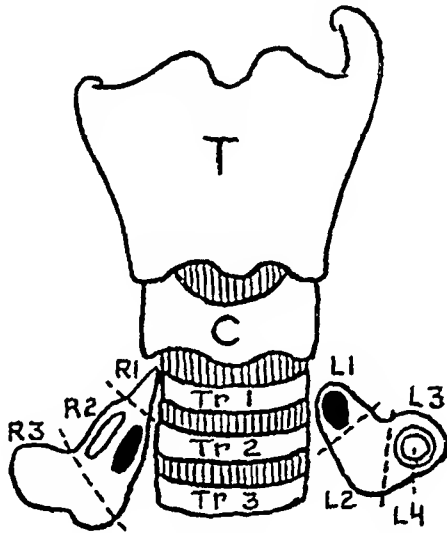


Fig 2—Diagram of neck structures in case of lingual goiter

Section R2 (midportion containing cysts) was a parathyroid gland. I interpreted it as such because of the characteristic arrangement of the cells in cords interspaced by blood-filled sinusoids.

Section R3 (distal end) showed no glandular elements. Areolar and adipose tissue and vessels were evident.

Left L-Shaped Body (fig 2) Section L1 (proximal tip) was a characteristic parathyroid gland. The cordlike arrangement of the cells, the intervening sinusoids and the characteristic general arrangement convinced me that this was one of the parathyroid glands.

Section L2 (midportion) showed no glandular elements and was composed of areolar and adipose tissues, it also contained vessels.

Section L3 (distal tip containing cyst) again showed alveoli which were compatible with embryonic thyroid alveoli. Again, however, I regarded this tissue as having originated from the branchial epithelial bodies. From which ones, it is impossible to ascertain.

Section L4 ("rice body" from the cyst in L3) was colloid and had no particular cytologic characteristic.

I believe that the thymus, thyroid and parathyroid glands are definitely accounted for in these sections. The unusual alveoli and glandular elements in sections R1 and L3 may have been derived from the so-called "lateral thyroid anlagen." This derivation is by no means clear. It would be safe, however, to call them derivatives of the branchial epithelial bodies. I regard the cysts as having originated from branchial epithelial body anlagen.

The absence of other thyroid tissue in cases of lingual thyroid is probably common in view of the frequency of myxedema following removal of the lingual growth. According to Ungermann,¹ six of eight patients examined at autopsy had no thyroid tissue other than that found in the lingual growth.

Dr. Curtis had an analysis made of the lingual thyroid tissue in his laboratory by Francis J. Phillips and reported as follows:

The gland as removed from the preservative fluid weighed 3.6 Gm. It was desiccated in a vacuum desiccator at 40 C. The weight after desiccation was 0.476 Gm. Three determinations made on this desiccated gland showed an iodine content of 31.6 mg. per hundred grams of desiccated gland. The preservative fluid from the gland was likewise analyzed in triplicate. The average result of these analyses was 0.168 mg. per hundred cubic centimeters of fluid. Therefore the entire gland contained 0.25 mg. of iodine.

This amount is to be contrasted with that found in the normal thyroid, which is about 2 mg. of iodine per gram of dry weight, or from 10 to 15 mg. in the entire gland.² As a control a portion of skeletal muscle from the dwarf was also analyzed, and no detectable amounts of iodine were found. It is of interest that in the thyroid from a typical achondroplastic dwarf I was unable to find any iodine by a method capable of detecting 0.05 mg. of iodine,³ whereas in the thyroid from a giant examined by Bassoe⁴ I found 62.9 mg. of iodine.

No important changes were found in the brain, which was examined by Dr. Percival Bailey. The hypophysis, however, was greatly altered, consisting of a soft, thin-walled cyst, which collapsed when the brain was removed. The sella turcica was slightly enlarged but otherwise normal. There was no evidence of change in the floor of the sella.

When examined microscopically the hypophysis was represented only by the collapsed wall of the cyst as obtained at the autopsy and consisted in part of the capsule surrounding the gland. The structure was not a definite cyst with a wall lining the cavity but rather a fibrous wall consisting of the capsule of the hypophysis and the adjacent membranes, within which lay fragments representing different elements of the hypophysis. There were many small masses representing the anterior lobe, in which the cells seemed to be flattened into rows by pressure. There was an abundance of posterior lobe tissue, and the structures of the pars intermedia were recognizable in close relation to the pars nervosa. The cellular elements of each of these segments showed the histologic characteristics of a normal

1 Ungermann. *Virchows Arch f path Anat* **187** 58, 1907

2 Wells, H. G. *Chemical Pathology*, ed 5, Philadelphia, W. B. Saunders Company, 1925, p. 690

3 Hektoen, L. *Am J M Sc* **125** 751, 1903

4 Bassoe, P. *Tr Chicago Path Soc* **5** 231 1903

pituitary as usually seen in bodies dead for several days. The impression was that the cyst had been produced in the anterior lobe by simple softening. There was nothing indicating any sort of neoplastic process or of hypophyseal duct cysts. There was no evidence of inflammation, either acute or chronic.

The skeleton was examined with roentgen rays by Dr. Paul C. Hodges, who reported the following results:

Roentgenograms of the pelvis and the lower part of the abdomen were taken with the subject in the supine position on grid visualization from the upper lumbar

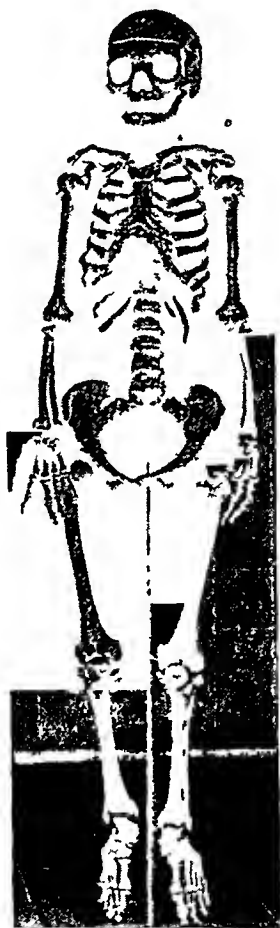


Fig. 3—Skeleton of dwarf with lingual goiter, showing absence of usual cretin characters, slight relative shortening of limbs, and well developed female pelvis.

part of the column halfway to the knees. I suspected a congenital abnormality of the upper sacral and lower lumbar segments. I looked for unfused epiphyses but found none. The bones were small. The pelvis was somewhat deformed. The femoral heads were slightly mushroomed, and the necks were short. A solitary bony island was present in the neck of the left femur. Some of the vessels were calcified. Roentgenograms of the upper part of the trunk were taken with the subject in the supine position on grid visualization of both shoulder joints, the elbow joints, most of the ribs and the vertebrae from the sixth cervical to the fourth lumbar. No lesions were seen. There were no unfused epiphyses. The

teeth were all missing. The bones, of course, were very small for a subject of the patient's age. The roentgenogram of the skull was taken with the left side against the film. The region of the pituitary gland showed the effect of the removal of bone at autopsy. The bones of the skull were rather thick. The teeth had all been removed. The roentgenogram of the knee was taken with the lateral surface against the film. There was marked wasting of the soft tissue. Calcification of the arteries was present above and below the knee. The articular ends of the femur and tibia had a rectangular blocklike appearance, and the articular cartilage appeared to be thin. No osseous lesion and no unfused epiphyses were observed. The roentgenogram of the left ankle was taken with the lateral surface against the film. There was generalized osteoporosis which may have been due to disuse or to senility. The peripheral vessels were calcified. There were no unfused epiphyses. In the left hand, wrist, forearm and elbow no lesions were observed except for wasting, calcification of the blood vessels and smallness. In the right hand and forearm, of which roentgenograms were taken with the palm down, there was evidence of an old Colles fracture of the distal end of the radius and of the ulnar styloid. Except for this, the appearance was the same as in the corresponding parts on the left.

After the skeleton had been separated from the soft tissues and mounted it was found to be characterized by the small diameter and delicate appearance of all the bones (fig 3). Relative shortness was evident in the extremities as in the entire body. The old healed fracture of the right radius was the only recognizable deformity. There was no evidence that the epiphyseal lines had failed to close, and there was no suggestion of rachitic deformity. The alveolar processes of the jaws had been entirely absorbed.

COMMENT

As dwarfism may result from either thyroid or hypophyseal deficiency, it is difficult to determine the relative rôle played by each condition in this case, as a history of the early life of the patient and metabolic or other clinical studies are lacking. As Erdheim⁵ has pointed out, both the thyroid gland and the hypophysis are necessary for normal endochondral ossification, for the presence of thyroid tissue alone when the hypophysis is destroyed or the presence of the hypophysis alone with the thyroid destroyed does not suffice to produce normal endochondral ossification. Erdheim believed the influence of the hypophysis to be greater than that of the thyroid gland, since in hypophyseal dwarfs the lines of ossification remain open in more bones and up to a later age than in cretins, however, cretins do not usually live very long, while a total loss of the hypophysis may permit of a longer life. Essentially, the hypophysis and the thyroid gland have the same effect on the three phases of endochondral ossification. Peritz stated that the growth tendency at birth seems to be adequate to carry the development of stature only to from 130 to 140 cm, beyond this height the influence of the hypophysis is required.

Athyreosis alone, despite a normal-appearing hypophysis, may lead to marked dwarfism. Janney⁶ says that some of the most severe cases of dwarfism known have occurred among cretins. These dwarfs usually

5 Erdheim. Beitr. z. path. Anat. u. z. allg. Path. **62** 303, 1916

6 Janney, in Barker, Lewellys F. Endocrinology and Metabolism, New York, D. Appleton & Company, 1922, vol. 1, p. 391

show a low degree or almost complete lack of intelligence, as contrasted with the good intelligence usually exhibited by the proportionate dwarfs, and cretins usually show a marked underdevelopment of the genital system

According to Paltauf⁷ the skeleton of the cretin is characterized by short, thick tubular bones which exhibit a coarseness in surface structure. The epiphyseal ends tend to be thick, low and flat, so that the bone scarcely possesses a true neck. The head of the femur is flattened and the neck short, producing the usual waddling gait. The base of the skull is typically short, as Virchow described it, with complete synostosis between the occipital and sphenoid bones, and the anterior and posterior sphenoid centers are fused. These fusions produce the typical broad, flat nose with depressed root usually exhibited by cretins as well as by achondroplastic dwarfs.

Although the patient under discussion undoubtedly had defective thyroid function and presumably was a cretin, the anatomic observations were not typical of cretin dwarfs as described by Paltauf, for the skeleton was well formed and graceful, the base of the skull was not shortened, and the facies generally observed in cretins was not present except for an increased width of the glabella, which measured 31 mm from orbit to orbit. At the advanced age of this woman the lines of ossification had completely closed and seemed normal. While in hypophyseal dwarfs the epiphyseal lines remain open for a long time, they do eventually close, as in the remarkable case reported by Priesl,⁸ that of a dwarf measuring 132 cm who lived to be 91 years old, at autopsy the epiphyseal lines were found closed, the anterior hypophyseal lobe was a thin-walled cyst as in my case, but the thyroid was present in the usual site, weighed 15.9 Gm and showed a normal histologic picture according to the age of the dwarf.

As far as is known there had been no myxedema in the case here reported despite the scanty amount of thyroid tissue present. While the extremely atrophic condition of the skin at the time of death suggested the previous existence of some trophic deficiency in the skin, premature senile changes of the skin (geroderma) have been described in hypophyseal dwarfs.

The skeleton in this case showed no severe disproportion. Although the arms and lower extremities were short in proportion to the trunk, this was not extremely marked, and did not resemble the disproportion observed in achondroplastic dwarfs. In this respect the conformation resembled more that of the hypophyseal dwarfs, who are classed with the proportionate dwarfs, than that of the cretin dwarfs who are classed with the achondroplastic and rachitic among the disproportionate dwarfs. However, the limbs were definitely short in proportion to the trunk for, as can be seen from the photograph, the finger-tips reached but little below the great trochanters. The lower extremities bore about the same relation to the trunk as did the upper extremities. There were no traces of rachitic changes in the bones.

7 Paltauf, Arnold. Ueber den Zwergwuchs in anatomischer und gerichtsarztlicher Beziehung, nebst Bemerkungen über verwandte Wachstumsstörungen des menschlichen Skelettes, Vienna, Holder, 1891.

8 Priesl, A. Beitr. z. path. Anat. u. z. allg. Path. **67** 220, 1919, Wien med. Wchnschr. **80** 589, 1930.

There seem to have been few observations of dwarfism in association with lingual goiter, which is of itself an infrequent condition, and none of the cases previously reported had been studied post mortem. In 1914 Asch⁹ was able to collect but ninety-five cases of lingual goiter recorded in the literature to that date, of these only 12 per cent were in males. In his discussion of the literature Asch made no mention of dwarfism as associated with lingual goiter, although a few such cases have been reported. Walther¹⁰ reported the operative removal of a lingual goiter from a *zweighalft kleine Frau* of imbecile appearance, who had lost the power of speech at 12 years because of the lingual tumor and had been confined in an institution thereafter. But he gave no measurements or other details and did not state the results of the operation. This case apparently was in some respects like the one here reported.

Another similar case has been reported by de Boncourt¹¹. This report concerned a dwarfed female cretin 122 cm tall, aged 37, with the apparent mental development of a child of 10, who had much difficulty in speaking and some trouble in breathing. The goiter was removed, but further events were not reported. Other cases of retarded growth associated with lingual goiter, but also without autopsy observations, have been reported by Urban,¹² Harvey,¹³ Krassnig¹⁴ and Rebattu.¹⁵ All these dwarfs with lingual goiters were females.

In 1921 Kohl¹⁶ collected ninety-three cases of lingual goiter—of which eighty-three occurred in females—often giving brief abstracts of the cases in addition to the titles but no further mention of dwarfism could be found among these reports.

Whether the dwarfism in the case under consideration was due entirely to the thyroid deficiency or partially or entirely to the hypophyseal abnormality cannot be determined. Against the assumption that that lesion was responsible is the character of the hypophyseal lesion, which seemed to be merely a central softening of unknown etiology. There remained an amount of anterior lobe tissue which according to its microscopic appearance might well have been adequate for the needs of the small body. Nor did the microscopic appearance suggest that this lesion was of sufficient age to correspond with the period of fifty-five years since the developmental stage of the subject. Simmonds¹⁷ has called attention to the fact that to cause dwarfism destructive lesions of the anterior lobe must occur during the years of growth, later in life they cause cachexia. In most of the few established cases of hypophyseal dwarfism the responsible lesion has been a cystic tumor of the hypophyseal duct as

9 Asch, R. Deutsche Ztschr. f. Chir. **130** 593, 1914

10 Walther. Beitr. z. klin. Chir. **77** 116, 1912

11 von Chamisso de Boncourt, A. Beitr. z. klin. Chir. **19** 281, 1897

12 Urban, Karl. Zentralbl. f. Chir. **50** 701, 1923

13 Harvey, F. Brit. J. Surg. **13** 746, 1926

14 Krassnig, Max. Ztschr. f. Laryng., Rhin., Otol. **12** 113, 1924

15 Rebattu. Presse med. **31** 904, 1923

16 Kohl. Schweiz. med. Wchnschr. **51** 361, 1921

17 Simmonds. Deutsche med. Wchnschr. **45** 487, 1919

described by Erdheim. However, in Priesl's 91 year old dwarf the anterior lobe of the hypophysis was described as consisting of only a thin membrane with scanty parenchyma rests, similar to the hypophysis in my case but differing in the presence of an osseous defect in the sphenoid with a persistent craniopharyngeal duct. Simmonds made the assumption, without any supporting evidence, that in this case the anterior lobe had been destroyed by embolism, but the presence of the persistent craniopharyngeal duct suggests the probability that this had something to do with the condition.

As Sternberg¹⁸ has pointed out, infantile dwarfs are not necessarily hypophyseal, for the proportionate dwarfs with hypothyroidism must be considered as an independent group of nanosomia infantilis. There is also a third group of dwarfs in whom both the hypophysis and the thyroid are normal or at least not responsible for the defects of growth, and these persons are characterized by hypoplasia of the sex glands, whereas in dwarfs with hypophyseal and thyroid deficiency the sex glands atrophy because of endocrine deficiency. Obviously the hypoplasia of the sex glands is not responsible for dwarfism, since eunuchs are tall. There seems to be no constant relation between the state of the hypophysis and that of the thyroid in cretinism according to the reports in the literature (Falta). When the thyroid is removed there is a tendency for hyperplasia of the anterior lobe, but a report of a cystic condition such as that seen in my case could not be found as a sequel of thyroidectomy.

Perhaps the case most like the one under consideration in respect to the hypophysis is that reported by Kraus and Holzer¹⁹ of a female cretin dwarf, 40 years old, 115 cm tall, in whose hypophysis was found a large irregular cyst involving one third of the anterior lobe, in which the parenchyma had been replaced by a thin colloid-like fluid containing many basophil cells. This cretin had a thyroid in the normal site showing much fibrosis and adenomatous areas. Death followed a cesarean section with delivery of a normal child. All the epiphyseal lines were closed, and the skeleton was well proportioned except for the relative shortness of the lower extremities. However, in this case the large skull and sunken nose of cretinism were present. Kraus and Holzer considered that the hypophyseal cyst formation was probably not responsible for the dwarfism, they believed that it was relatively recent and secondary to the thyroid deficiency, and they called attention to the fact that Bayon and von Stefanoff observed an increase in the hypophyseal colloid in cases of thyroid aplasia. Although their case is more definitely one of typical cretinism than the case reported here the two cases together indicate that cystic changes in the hypophysis may result from a high grade of thyroid deficiency.

All things taken together, it seems probable that this case is primarily one of dwarfism due to hypothyroidism, in which the functional thyroid tissue is reduced to a minimum amount in a greatly degenerated lingual goiter, there having been no formation of thyroid tissue at the normal sites. As is usual in these cases, the parathyroids are normal and the suprarenal glands, while very small, do not show definite abnormalities.

18 Sternberg, C. Beitr. z. path. Anat. u. z. allg. Path. **67** 275, 1919.

19 Kraus and Holzer. Virchows Arch. f. path. Anat. **251** 253, 1924.

Apparently sexual development ceased at about puberty, a few ovulations perhaps having taken place. However, it is to be noted that the skeletal changes were not those of typical cretinism, and in some respects the condition resembles "pituitary infantilism" as described by Berblinger,²⁰ characterized by a defective development of the anterior lobe of the hypophysis and hypoplasia of the genital organs, with normal development of the other endocrine glands. In these cases adiposity is absent because of the lack of involvement of the brain.

SUMMARY

The case here reported is that of a mentally substandard female dwarf, 72 years old, 49 inches (124.5 cm) tall, weighing about 40 pounds (18.1 Kg), who had been in institutions for the care of the incompetent and impoverished for thirty-seven years and on whom no clinical study had been made. No history of her youth or family could be obtained. Death was due to an impaction of food in the larynx, trachea and bronchi, perhaps because of the obstruction to regurgitation caused by a lingual goiter, measuring 2.5 by 2.3 by 1 cm. No other thyroid tissue could be found in the neck or elsewhere. This lingual thyroid consisted mostly of necrotic fibrous tissue, apparently the result of organization of old hemorrhages and involuted thyroid tissue. Only at the periphery of the nodule were traces of recognizable thyroid tissue found, and this was very atrophic. In it was found but 0.25 mg of iodine, as compared with from 10 to 15 mg, the amount present in a normal thyroid. The chief other observation was a cystic degeneration of the anterior lobe of the hypophysis, of unexplained etiology and undetermined significance. Presumably this case is one of dwarfism due to hypothyroidism associated with incomplete cretinism, it is improbable that the hypophyseal cyst was responsible for the dwarfism, although the hypophyseal influence cannot be excluded.

20 Berblinger, W. Beitr. z. path. Anat. u. z. allg. Path. **87**: 233, 1931.

Laboratory Methods and Technical Notes

A SIMPLE APPARATUS FOR FIXATION OF LUNGS IN THE INFLATED STATE

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Ordinarily, the introduction of fixing solution (formaldehyde or Kaiserling I¹) into the bronchi inflates the lungs satisfactorily, but unless the inflated condition is maintained evenly only the dependent portions (containing the greater part of the fixing solution) are permanently inflated, the remaining parts becoming fixed in partial inflation of uneven character. A method employing negative pressure for maintaining complete uniform inflation within physiologic limits has been worked out in this laboratory and has proved practicable for most purposes. The principle of this method depends on the maintenance of a certain degree of inflation of the lung for a number of hours, during which time the fixative may act.

The lung to be inflated must be removed from other structures, viz., the heart, pericardium and diaphragm, with the least possible injury to its surface, either by instruments or by the tearing of adhesions, in order to avoid leaks. As much of the bronchus as possible should be left on the lung, though a length of 1 cm may suffice. One end of a short metal tube is inserted into the bronchus as far as it will pass easily, as shown in the figure. The tube should be about $\frac{1}{2}$ inch (1.27 cm) in diameter and from 2 to 4 inches (5.08 to 10.16 cm) long, its other end should be threaded (*J*) for connection with a metal joint (*F*). To attach the lung to this tube it has been found necessary to adopt the following procedure. The stump of bronchus is grasped at one part of its edge by a narrow clamp, which is held in the same hand as the metal tube previously inserted, this prevents the tube from slipping out of the bronchus. A chain of elastic rubber bands looped end to end to make a total length of from 18 to 24 inches (45.7 to 60.9 cm) is used to tie the bronchus on the metal tube (*K*). As this is wound about the bronchus constant tension is maintained, the clamp should not be included in the winding and may be released after a number of turns. By means of the constant elastic tension a surprisingly satisfactory joint is made between the bronchus and metal tube, and, in our experience, this has never slipped or leaked.

An ordinary large bell jar (*G*) is employed for the negative pressure chamber. Since it is made of glass, the stages of inflation of the lung may be studied, and roentgenograms may be taken at any stage. The jar is supported in a wooden base (*I*) with a concavity made on the lathe to fit the convexity and knob of the bell jar exactly. The cover of the chamber is made of a broad, thick disk of wood (*D*) with a circular groove (*E*) cut near its edge to fit easily over the rim of the jar, the groove should be wide enough to permit the cover to be removed

From the Laboratories of the Mount Sinai Hospital

1 This solution consists of 30 Gm of potassium acetate, 10 Gm of potassium nitrate, 750 cc of distilled water and 300 cc of a solution of formaldehyde.

easily. An air-tight seal is made by partly filling the groove with petrolatum just before it is applied to the jar. An opening is cut directly in the center of the cover for a large-sized rubber stopper (*C*), through which, in turn, a threaded metal tube (*B*) is inserted snugly and made to project far enough below the under surface of the cover so that the metal joint (*F*) may be screwed on. The lung is attached to the joint by means of the other metal tube (*J*) previously described, after the bronchus has been tied on. Another opening is cut in the cover near one edge to admit a small glass Y-tube (*A*), one arm of which is connected with a suction pump, the other with a simple water manometer (*H*) by means of a flexible rubber tube. The Y-tube with its connections is inserted after the cover with the suspended lung is in place, and is removed when the cover is to be taken off.

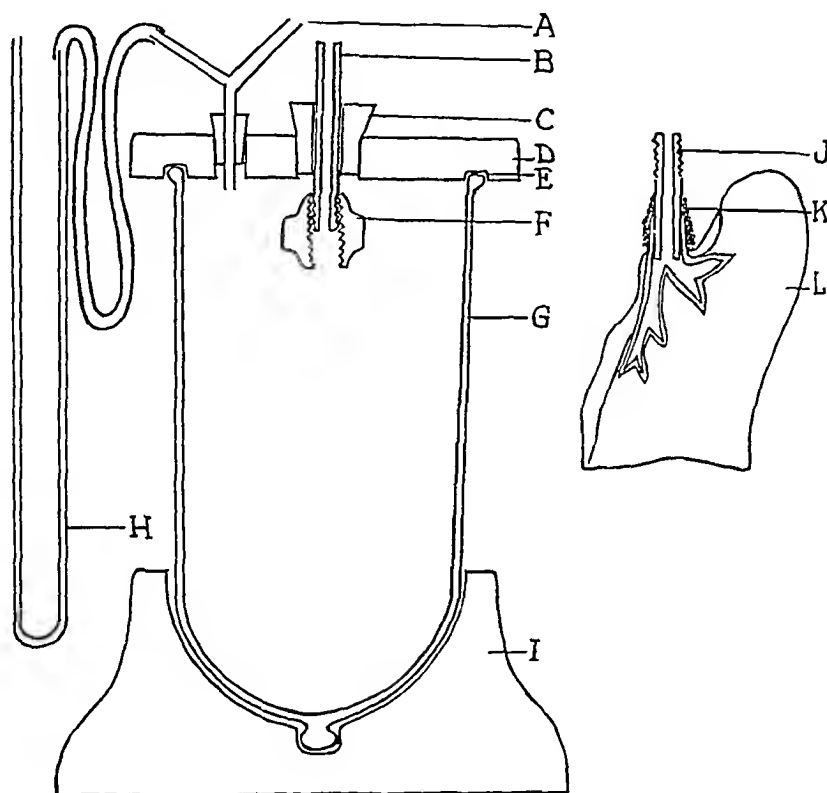


Diagram of bell jar and connections employed in maintaining inflation of lung to be fixed. *G* indicates the bell jar, which is supported in the wooden base *I* and is covered with a broad thick disk of wood *D*, with a circular groove *E*. *C* is a rubber stopper, through which a threaded metal tube *B* is inserted and made to project far enough below the surface so that the metal joint *F* may be screwed in. The lung is attached to the latter by means of the tube *J*, one end of which is inserted into the bronchus, which is tied on the metal tube *K*. *A* is a small glass Y-tube, one arm of which is connected with a suction pump, the other with a simple water manometer *H* by means of a flexible rubber tube.

With the lung in place, suction is applied (from 10 to 20 cm of water) until the inflation reaches its maximum throughout, the entire lung should expand uniformly, unless pathologic alterations are present or the bronchus is blocked by improper fitting of the metal tube. Illuminating gas may now be run into the

bronchus through *B* to fix the hemoglobin of the lung a permanent red fixing solution (Kaiserling I) is then introduced into the bronchus by means of a funnel joined to *B*, this is added slowly until a total of from 2 to 3 liters has been used. Ordinarily, a considerable amount is lost through surface tears and through the blood vessels of the hilus, and a slight amount through surface transudation from the pleura. While the fixing fluid is being added all leaks are rendered temporarily air-tight, so that a marked rise in negative pressure usually takes place, requiring adjustment of the suction pump repeatedly. When the addition of fixing fluid is completed, it is necessary to regulate the suction until a stable reading for negative pressure is reached. Suction should be left on from twelve to eighteen hours.

In the presence of rents in the pleura, it may be possible to produce a serviceable seal by searing the torn surface with a hot iron until it is perfectly dry and applying liquid adhesive reenforced with cotton wool, this must be permitted to dry completely.

When a lung fixed in this manner is opened it appears somewhat dry on cut section, its color is delicate salmon pink, the normal alveoli are distinct, and the bronchi and bronchioles appear as smoothly rounded tubes which retain their shape. If a portion of lung tissue is squeezed, even to the thinness of paper, it still retains its capacity to reexpand, and if placed in water swells and regains its former appearance immediately.

This method is not suited to diseased lungs in which it is desired to maintain atelectasis. On the other hand, it is ideal for such conditions as bronchiectasis, bullous emphysema, suppurative pneumonia and tuberculosis.

Microscopically, the alveoli appear unusually well inflated and almost emphysematous, yet with intact walls, even when inflated with extremes of pressure. The finer architecture of the lung is much more distinct than is usually seen, even in lungs removed under ideal circumstances from healthy persons dying suddenly. It is possible to distinguish the complete ramification of the bronchioles and alveolar ducts in almost diagrammatic perfection. The alveolar septums appear thinner than usual and somewhat elongated, their capillaries show corresponding changes. Edema fluid has a foamy appearance on section, as though churned violently with the air used for the inflation. Pneumonic exudate appears looser than usual, and the inflamed alveolar septums are more clearly defined, often showing leukocytes in passage from capillaries into alveolar interstitial spaces and alveolar lumens. The inflamed alveolar septums often appear slightly edematous, revealing a distinct interstitial space containing fibrin and blood cells. The interstitium is not apparent in uninflamed alveoli, even when greatly congested. The lymphatics, in general, are rendered more prominent, with prolonged inflation and the use of large amounts of fixing fluid they may be emptied of exudate or tumor cells. (This observation may possibly possess some significance in relation to the physiology of the circulation of lymph within the lung.)

By means of overdistention with fixing fluid and prolonged inflation under high negative pressure, a unique microscopic picture is produced. The alveolar architecture is preserved, the alveolar septums, however, are extremely thin and stretched practically straight in places, so that

many septums lie flat instead of being perpendicular to the surface of the slide, appearing as narrow ribbons. Wider ribbons are seen, with sections of greater thickness (e g, 20 microns). It is thus possible to study the surface of the alveolus in strips. In our experience with this method we have never found pores of Cohn² to exist in normal alveoli. Elastic fibers are numerous and alveolar epithelial cells are seen singly in places lying on the surface. There is no evidence of non-nucleated squames (von Kolliker).

2 Pores of Cohn, or Cohn's stomas, are minute apertures in the walls of the pulmonary alveoli. Whether they actually exist preformed, permitting the passage of air, edema fluid or fibrinous exudate from one alveolus into another, or whether they are artefacts has not been determined.

General Review

EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS AND ITS RELATIONSHIP TO HUMAN ARTERIOSCLEROSIS

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I INTRODUCTION

In the experimental study of arteriosclerosis in animals the most painstaking efforts have been directed toward the production of arterial lesions capable of really close comparison with those of human arteriosclerosis. The lesions produced in the arteries of rabbits by cholesterol feeding represent the nearest approach to success in this direction. It is well known that arterial lesions can be produced quite easily in rabbits by a variety of other methods, but with these other methods the alterations in the walls of the vessels are confined almost entirely to the media. The intima is only secondarily affected, and the changes in it are relatively insignificant. Slight intimal thickenings of a purely fibrous character may be found but not with any regularity. These experimental lesions of the arteries can hardly be said to resemble those of ordinary human arteriosclerosis, in which thickening of the intimal layer is perhaps the most constant and conspicuous feature. Moreover, in human arteriosclerosis, the intimal thickenings are seldom composed of fibrous tissue alone, they are almost constantly the sites of more or less abundant accumulations of various lipid substances. Intimal lesions of this nature, so familiar in human arteries, have been very closely imitated in the cholesterol feeding experiments which I propose to review in this paper. A complete and exact experimental reproduction of human arteriosclerosis in animals has not yet proved possible.

In rabbits, the feeding of large quantities of cholesterol or of foods rich in cholesterol results in the development of widespread lesions of the arteries. These lesions are characterized by a thickening of the intima and by abundant accumulations of lipoids^a in the intima and the inner layers of the media. I have chosen to call this experimental disease of the arteries "experimental cholesterol arteriosclerosis." Cholesterol feeding as a method of producing fatty lesions in the arteries of rabbits had its origin in some experiments reported in 1908 by Ignatowski, who found the typical arterial lesions in rabbits which had been fed for some time with eggs and milk. The general resemblance between these lesions and the lesions typical of human arteriosclerosis was immediately recognized and aroused great interest. The obvious opportunities for investigation offered by the new experimental method gave rise to numerous studies of experimental cholesterol arteriosclero-

(a) Following Peters and Van Slyke,¹¹⁹ I shall apply the term "lipoid" to all those substances in the organism which, in their general chemical and physical properties and especially in their solubilities, resemble the true fats. The latter are included also. The words "fat" and "fatty," unless otherwise specified, will be used in a loose sense to indicate especially those lipoids which can be stained with ordinary "fat stains," such as sudan III.

sis It is chiefly to the results of these studies and to a consideration of their significance in relation to human arteriosclerosis that this paper will be devoted

It should be mentioned at once that the effects of cholesterol feeding are not confined to the arteries, the lipoids accumulate also in various other tissues and organs throughout the body It is not the intention here to describe the extra-arterial changes in detail or to enter into a discussion of their significance except so far as they are concerned in the interpretation of the lesions in the arteries The possible applications of the experimental data to problems other than that of arteriosclerosis have been discussed fully by Verse,¹⁶⁵ Kawamura⁷⁰ and Chalataw³² These applications are really rather limited Certainly it is safe to say that the most important inferences which have been drawn from the cholesterol feeding experiments are those relating to the etiology and pathogenesis of arteriosclerosis in man

Even in the early lesions of human arteriosclerosis, the presence of lipoids in the intima can be easily recognized by routine histologic methods, and for this reason, the rôle of fatty substances in the development of arteriosclerosis has always occupied a prominent place in discussions of the pathogenesis of the disease However, there are other alterations in the arterial walls which are not so readily demonstrated under the microscope and which may be even more important than the accumulation of lipoids It has never been satisfactorily established from the study of human material that the lipoids play the major rôle Whether they represent cause or effect is still a matter of controversy On this question, more than on any other, the experimental results have been brought to bear as presumptive evidence of the great importance of the lipoids in the etiology and pathogenesis of human arteriosclerosis

My interest in the cholesterol feeding experiments was aroused by the claims of many authors that experimental cholesterol arteriosclerosis in rabbits is virtually identical with human arteriosclerosis In my own experiments certain features of the pathologic picture seemed to be rather inconsistent with such a conclusion However, a more complete and thorough examination of the literature showed that my results differed in no essential respect from those of previous investigators The discrepancy lay in the fact that the points of difference between human arteriosclerosis and the experimental disease have been almost always completely subordinated to the features of similarity

The experimental data which have now accumulated have come to assume considerable importance On the assumption of identity between experimental and human arteriosclerosis, the experimental results are frequently cited as evidence in support of a modification of the so-called "imbibition" or "infiltration" theory of human arteriosclerosis This

theory, which was first proposed many years ago,^b is offered in a somewhat different form as an explanation of the development of experimental cholesterol arteriosclerosis. It is assumed that the modified theory may be applied quite as appropriately in the explanation of the development of arteriosclerosis in man. The modification consists chiefly in the great emphasis which is laid on hypercholesteremia as a factor of primary etiologic significance in human arteriosclerosis. Such unreserved acceptance of the experimental data as evidence capable of direct application in the human being has led to inferences of far-reaching importance, which have attained widespread credence and popularity through the extensive writings of numerous authors. It seemed worth while, therefore, to examine rather carefully the experimental work on which these ideas are based, with the purpose of determining what facts have been established and of evaluating their importance as applied to the solution of problems of arteriosclerosis in man.

II ORIGIN AND DEVELOPMENT OF CHOLESTEROL FEEDING EXPERIMENTS

In 1908 Ignatowski,^c studying the effects of high protein diets on rabbits, noticed the occurrence of fatty plaques on the intimal surface of the aorta in some of his animals, as well as fatty accumulations in the liver and kidneys and marked enlargement of the suprarenal glands. The feedings had consisted of meat, eggs and milk, and he attributed the pathologic changes to the effect on a herbivorous animal of a diet rich in animal proteins. In the following year, Starokadomsky^d studied the lesions in the arteries more carefully and described them fully. He had given his rabbits a diet of eggs and milk, and he, too, believed the lesions to be due to the toxic effect of the proteins in the diet. However, the experiments of Stuckey,¹⁵² reported in 1910, threw some doubt on this point. He fed rabbits with milk, egg-white, egg yolks and meat juice in various combinations and found that the diets containing egg yolks produced marked fatty changes in the aorta and some of its large branches, while milk, egg-white and meat juice alone had no damaging effect on the arteries. Stuckey¹⁵³ was later successful in producing identical lesions by feeding brain tissue. These findings, together with other considerations, led to the idea that the lesions in the arteries and elsewhere were not due to the protein fraction of the diet but rather to some lipoid constituent common to egg yolks and brain tissue.

(b) References 15, 16 and 124 in bibliography

(c) 64, 65, 66

(d) 147, 148

The experiments of Wesselkin^e with the feeding of lecithin, although only 1 animal was given a really large amount, strongly indicated that this substance was not responsible for the pathologic alterations. Stuckey¹⁵³ had already shown that diets containing neutral fats and fatty acids were ineffective in producing the arterial lesions. It remained for Anitschkow and Chalátow,^f in 1913, to demonstrate that the feeding of pure cholesterol^g dissolved in oil could produce in rabbits lesions identical with those described by previous investigators. Independently and almost simultaneously, Wacker and Hueck^h reported that pure cholesterol in solid form added to the ordinary food of rabbits could produce the lesions, and they showed further that it brought about a marked increase in the cholesterol content of the blood, an increase which was dependent for the most part on an elevation of the cholesterol esters.

Since that time similar experiments with various modifications have been carried out on rabbits by numerous investigators who have studied and described fatty accumulations in a variety of situations throughout the body. This work has demonstrated that the lipoids, which are for the most part anisotropic, are deposited first in the cells of the reticulo-endothelial system in the liver, spleen, lymph nodes and bone marrow. The cells of the suprarenal cortex and the central cells of the liver lobules become heavily loaded with lipoids. Later, fatty deposits occur in the arteries, in the valves of the heart and in the veins, in the skin, subcutaneous tissue and tendons, in the mucosa and submucosa of the bile ducts, gallbladder and stomach, in the interstitial tissue of the kidneys, and in the eyes, simulating the arcus senilis.

The effects of cholesterol feeding have been studied also in a variety of animals other than the rabbit, but attempts to produce arterial lesions in other animals by this method have met with limited success. Bailey¹⁹ in 1915 reported the production of experimental cholesterol arteriosclerosis in guinea-pigs, and his results have since been confirmed by several other investigators. It is now clear enough that cholesterol-rich diets are capable of producing arterial lesions in guinea-pigs as well as in rabbits, but there has been no convincing demonstration of a similar effect in any other animal.

The literature bearing on the changes produced in various organs and tissues by cholesterol feeding has been well summarized by Schon-

(c) 178, 179

(f) 4, 13

(g) This and subsequent references to "pure cholesterol" indicate preparations of "cholesterol" supplied by commercial firms or manufactured by the experimenter. It is not to be inferred that such preparations consisted of a single pure chemical. In all probability they contained two or more sterols of which one was almost certainly ergosterol.¹¹⁹

(h) 166, 167

heimer¹³⁵ More comprehensive publications in this field are those of Kawamura⁷⁰ and Chalutow³² The literature dealing especially with experimental cholesterol arteriosclerosis has been summarized briefly by Schultz,¹³⁸ and quite recently Anitschkow¹² has written a full review of the subject from the Russian point of view

III METHODS

A variety of cholesterol-rich diets have been administered in various ways by different investigators for the production of experimental cholesterol arteriosclerosis Egg yolks have been the most commonly used of cholesterol-rich foods,¹ but brain tissue,³ liver⁴ and hydrous wool fat¹ have been used with success These substances were mixed with the ordinary food (in the case of liver, as a dry powder), or were thinned out by mixture with some liquid and administered by means of a stomach tube After the discovery that cholesterol alone would produce the same results, it was commonly employed Solid cholesterol has been given mixed with oats or bread^m or administered in capsules³⁶ Others have used an oily solution of cholesterol, with which meal or bread was saturatedⁿ Still others have introduced the cholesterol in oily solution directly into the stomach by means of a stomach tube,^o and this seems to be the method of choice A variety of mediums for the solution or suspension of cholesterol have been tried with success Sunflower-seed oil,^p cottonseed oil,^q olive oil^r and 5 per cent sodium oleate¹⁰⁰ all seemed to be suitable, and none of them alone produced changes in the arteries The cholesterol content of the preparations ranged from 2 to 7.5 per cent

The daily administration of the cholesterol ration through a stomach tube seems to be the most convenient method The only valid objection to the use of a tube is the occasional loss of an animal from perforation of the esophagus or stomach or from aspiration pneumonia However, both of these accidents can be avoided by the use of a soft tube, by practice in passing the tube and by care in cleaning it thoroughly before it is passed It has been pointed out that the insertion of the stomach tube might cause an increase in blood pressure Thollde¹⁵⁴

(1) 6, 7, 20, 42, 45, 67, 76, 152, 189, etc

(j) 153, 156, 157

(k) 38, 75, 76, 132, 156

(l) 34, 109, 141, 142, 143, 169

(m) 74, 131, 166, 167

(n) 20, 42

(o) 4, 5, 13, 45, 99, 100, 145, 189, etc

(p) 4, 5, 13

(q) 19, 20

(r) 45, 100, 167

has advanced evidence to the contrary, but with this possibility in view, some investigators have preferred to avoid the use of the tube and have given cholesterol or cholesterol-rich substances mixed with other foods. The objections to this method are the uncertainty as to how much cholesterol the animals actually ingest and the occasional difficulty encountered in inducing them to take the food.

Whatever method is chosen for the introduction of cholesterol, its administration must be continued for some time, and its total quantity must be considerable before the lesions in the arteries make their appearance. If solid cholesterol is fed, its administration must be continued for a longer time and its total quantity must be greater in order to produce the arterial lesions than is the case if it is employed in solution in oil or as it occurs naturally in egg yolks, hydrous wool fat, etc. Thus, Antschkow^s found the earliest microscopic changes in the aortas of his rabbits after from 30 to 45 days when a total of from 11 to 18 Gm. of cholesterol in oil had been given by mouth. The earliest gross alterations were seen at the end of from 55 to 78 days after a total of about 30 Gm. of cholesterol had been fed. Bailey²⁰ observed early changes in the aortas of his rabbits only after the administration by the oral route of 14.5 Gm. of cholesterol in oil in 36 days, or of 26 egg yolks in 30 days. On the other hand, Wacker and Hueck,^t who fed their rabbits with solid cholesterol were unable to find any changes in the arteries in less than 5 months, although the daily dose was 1.25 Gm., the total quantity of cholesterol therefore being in the neighborhood of 175 Gm. or more. Apparently the more rapid development of lesions after the feeding of cholesterol in solution is dependent on the greater ease with which it is absorbed in this form from the gastro-intestinal tract.

Some investigators have combined the administration of cholesterol-rich diets with other procedures designed to injure the arterial walls simultaneously. These procedures have included the use of the suspension method of Klotz,⁷² narrowing of the aorta by a loose ligature, injections of epinephrine hydrochloride or of certain toxic substances, or even direct injury to the wall of the aorta by cauterization. Others have modified the experiment by interference with the endocrine system with the object of altering the cholesterol metabolism in such a way as to favor or retard the accumulation of lipoids in the organs and arteries. These special methods will be mentioned later with their results.

At this point it may be remarked that the concentration of attention on the cholesterol content of the diet seems to have detracted from any serious consideration of its general nature. The diet, apart from the

(s) 4, 5

(t) 166, 167 .

added cholesterol, has usually consisted of foods commonly used for the maintenance of laboratory animals and, of course, has been the same as that given to control animals. Nevertheless, it seems much more desirable that the diet, except for its excessive content of cholesterol, should be a balanced one which is known to be adequate in all respects. In this connection, the work of Blumer and his associates²⁶ is suggestive, purporting to show that chlorophyll added to the diet retards the development of experimental cholesterol arteriosclerosis in rabbits. The effects of added substances other than cholesterol (e g, the other lipoids and the proteins in egg yolks, the oils used for the solution of cholesterol, etc) must also be taken into account.

A number of investigators have experimented with parenteral administration of cholesterol. By the use of intravenous injections of a preparation of cholesterol in 5 per cent sodium oleate, Klotz⁷³ was able to produce arterial lesions in rabbits which he described as corresponding to the early stages of the lesions occurring in the cholesterol feeding experiments. Dewey⁴⁰ reported similar results following intravenous or intraperitoneal administration of a colloidal suspension of cholesterol in water. These results, however, have not been confirmed by subsequent investigations¹¹ in which attempts to produce generalized changes in the arteries by means of injections of cholesterol have met consistently with failure. Various solutions of cholesterol and various routes of administration have been tried, but the parenteral introduction of cholesterol has not proved itself a method well adapted to the production of lesions in the arteries, perhaps because the doses of cholesterol are necessarily rather small or because the experiments have not been sufficiently protracted. Apparently cholesterol is absorbed rather slowly from the tissues even when it is dissolved in oil. Oily solutions can hardly be injected intravenously in large quantities, and colloidal suspensions of cholesterol in water, when injected into the veins, produce only a relatively slight and often temporary elevation of the blood cholesterol level. The increase occurs chiefly in the free cholesterol in the plasma, while the cholesterol esters remain practically unchanged. The cholesterol is rapidly removed from the blood and is found in the reticulo-endothelial cells. Evidently other lipoids are required to hold the cholesterol in the blood stream for any length of time in stable colloidal solution.

IV ARTERIAL LESIONS OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN RABBITS

(a) *Gross Appearance and Distribution*—The descriptions by numerous authors^v of the gross appearance and distribution of the

(u) 38, 46, 69, 70, 123, 140, 167, 189

(v) 4, 5, 20, 45, 66, 100, 131, 147, 148, 152, 153, 162, 164, 166, etc

arterial lesions which follow cholesterol feeding in rabbits show a general agreement, except when the experiment has been modified by procedures other than simple administration of cholesterol. The lesions of the arteries make their appearance first in the aorta but only after the liver, spleen, bone marrow and suprarenal glands have become loaded with lipoids. In the aorta, the earliest alterations are usually to be found in the arch about the mouths of the vessels arising from it or just above the aortic valve ring. They become visible on the intimal surface as minute yellowish-white opaque flecks which are raised slightly above the surrounding normal intima. At first these areas are not sharply limited but fade off gradually at their borders. However, with increasing size, the tiny thickenings often come to form well demarcated little nodular projections of rounded or irregular outline, which have a curious glistening appearance and a yellow or yellowish-white color. As the original lesions become larger, more and more yellowish opacities make their appearance in other parts of the arch, and they may be found also in the thoracic aorta. In the latter situation, they are more numerous on the posterior wall of the vessel and are usually described as tending to favor the areas about the mouths of the intercostal arteries. However, similar thickenings are often present on the sides or on the anterior aspect of the aortic wall. In some cases the lesions may be confined to the arch and thoracic portion of the aorta,²⁰ but the abdominal portion is frequently affected also, though to a lesser degree, and here, too, the areas about the mouths of branching arteries are more commonly the sites of lesions than areas elsewhere.

With increase in the dimensions of the individual nodules, which may reach the size of a split pea, adjacent nodules coalesce, forming large irregular plaques, or this may occur widely, especially in the arch of the aorta, so that large areas become involved. A rough, warty, yellowish surface results, and the thickening may be sufficient to cause a significant narrowing of the aortic lumen,¹⁰⁰ though later a dilatation of the vessel occurs. In the thoracic portion of the aorta, the confluence of a series of nodules may form irregular streaks, especially on the posterior surface of the vessel. In the abdominal portion, the irregular coalescence of individual nodules may produce a variegated picture. The most severe changes in the aorta are characterized by the appearance of fusiform dilatations usually in the arch, followed rarely by the formation of true aneurysmal sacs.⁸⁰ The elasticity of the vessel is appreciably reduced.¹⁰⁵

When the lesions in the aorta have become well established, and sometimes sooner, isolated fatty flecks may be found also in its larger branches, perhaps most commonly at points of bifurcation. The innominate, common carotid, subclavian, common iliac and femoral arteries are often the sites of lipid accumulations, but the renal arteries are

not frequently mentioned. Later, even the smaller branches of the arterial tree may show small nodular lesions. The coronary arteries of the heart are often attacked^w and to a lesser degree those of the spleen and mesentery. The arteries of the liver and kidney are even less affected, while the cerebral and retinal arteries always remain unchanged^x.

The main trunk of the pulmonary artery also shows fatty thickenings on the intimal surface soon after the earliest changes are found in the aorta. Small yellowish-white nodules first make their appearance in irregular distribution and later coalesce as in the arch of the aorta, forming a roughened and irregular surface. Isolated plaques may be found also in the large branches of the pulmonary artery and even in its finer ramifications.^y

(b) *Microscopic Appearance of Lesions in the Aorta*—Numerous descriptions of the microscopic appearance of these lesions in the arteries of rabbits are to be found in the literature,^z and regarding certain of their more obvious features there is no question. However, the lesions vary to a considerable extent in some respects even in apparently comparable series of experiments. This is evident in a comparison of a number of descriptions of the alterations in the arteries and is even more striking in a comparison of photomicrographs or drawings of the arterial changes. How much of this variation may be due to differences in the rate of development of the lesions with different daily doses of cholesterol or to natural differences between individual rabbits, or how much may depend on unknown and uncontrolled factors, can be a matter only for speculation at present. In any event, differences do exist. In the intima, they are to be found chiefly in the varying abundance of cells relative to the quantity of intercellular material and in the varying proportion between the numbers of the two main kinds of cells which appear. Often the prominent feature in the intima is the accumulation of large, foamy, fat-laden cells, which may compose the bulk of the thickening, while in other experiments the fibroblastic reaction is more pronounced. In still other cases, the intimal thickening is extremely poor in cells and is composed largely of homogeneous intercellular material, which I shall hereafter refer to as "ground substance," recognizing, of course, that it may exist in vivo as a fluid or perhaps in the form of a gel. In the media, the character of the lesions is much more constant, but the extent of the medial involvement varies considerably. Focal necrosis of the smooth muscle of the media followed

(w) 81, 84, 184

(v) 11, 12, 162, 164

(y) 20, 29, 45, 99, 100, 135, 179

(z) 4, 5, 20, 34, 45, 66, 76, 80, 89, 100, 126, 131, 135, 147, 148, 152, 153, 166, 169, 189, etc

by impregnation of these areas with lipoids may be a striking and early occurrence,^a or the appearance of the medial lesions may be delayed until the lesions in the intima are well developed

The lesions in the large branches of the aorta and in the smaller arteries have not been studied so thoroughly as those in the aorta itself. However, the lesions, wherever they occur in the arterial tree, seem to be of essentially the same nature. The description of the histologic changes in the arteries will therefore be confined to those occurring in the aorta.

Microscopic alterations in the aorta can sometimes be distinguished a month or more before the lesions become grossly evident, but the site of the earliest deposits of lipid material is a question about which there has been discussion. In the rabbit, the intimal layer of the aorta consists of a single layer of lining endothelium separated from the internal elastic lamina only by an extremely thin layer of intercellular ground substance in which an occasional spindle-shaped cell may be seen. The thin layer of ground substance first becomes swollen so that it is distinctly thicker than usual. It was in this layer of altered intercellular substance that Amitschkow^b and others^c observed the earliest deposits of anisotropic fat as fine droplets or granules, prior to the appearance of any fat-containing cells. Other authors^d concluded from their observations that the first appearance of lipoids was within cells of the lining endothelium or of the subendothelial layer. The burden of proof seems to rest with the latter group, for they all observed extracellular as well as intracellular fat. While the extracellular lipid material might have been released from necrotic cells, the possibility that it was first deposited in the intercellular material and later was taken up by cells cannot be excluded. From my own observations⁴⁵ I am convinced that anisotropic fatty materials may appear extracellularly in the intima in the altered subendothelial ground substance before any fat-containing cells are to be found. Indeed, the accumulation of lipoids in the greatly swollen subendothelial layer may become very abundant without the appearance of any considerable cellular proliferation. On the other hand, the extracellular lipoids may rapidly be masked by the advent of numerous cells which become laden with fat. It was found also that the inner layers of the media may be the site of the earliest deposits of lipid materials, as will be described more fully in a subsequent paragraph

At a slightly later stage, the thickenings of the intima of the aorta nearly always contain a considerable accumulation of cells, among which

(a) 20, 45

(b) 4, 5

(c) 126, 145, 164, 189, etc

(d) 20, 99, 100, 166

large, foamy, fat-laden cells are the most prominent. These foamy cells may sometimes show a palisade arrangement in rows vertical to the plane of the aortic surface,¹⁰⁰ but more frequently they are irregularly arranged and form a little heaped-up clump, which eventually becomes grossly visible as a tiny yellowish-white thickening on the intimal surface. The diameter of the individual cells ranges from 10 to 24 microns,^e and their outline may be rounded or polygonal or even somewhat flattened. In the smaller cells the cytoplasm forms only a relatively narrow rim about the nucleus, but in the larger ones it is voluminous. It is pale-staining and slightly basophilic, and shows a delicate reticular structure forming a spongy or honeycombed network which gives the cytoplasm a foamy appearance. The nucleus is usually centrally placed, small, round and deeply staining. In larger cells, however, it may be paler-staining or in some cases small and pyknotic, and it may be displaced from its central position. Some of the large cells contain two or three nuclei.^f It will be convenient to refer to the cells described in this paragraph as "foam cells."

The phagocytic foam cells have attracted special attention because of their exact resemblance in form and apparently also in function to the large fat-holding cells found in the intima of arteriosclerotic human arteries. The foam cells which accumulate in the intima in experimental cholesterol arteriosclerosis seem unquestionably to be macrophages. They resemble the large mononuclear phagocytes elsewhere in the animal body not only in appearance but also in their active phagocytosis of lipoids. Moreover, they take up colloidal dyes just as do the macrophages elsewhere,⁷⁰ so that there is really no reasonable doubt as to their identity. It is possible that the macrophages might migrate into the intima from the medial direction, but this possibility is practically excluded by the lack of foam cell accumulations in association with deposits of anisotropic lipoids in the media.^g There are those who believe that the macrophages enter the intima from the lumen of the artery. However, it appears quite possible that they may arise through the multiplication of wandering mononuclear phagocytes present in the intima prior to the appearance of lipoids. In any event, this is a possibility which has not been excluded.

In the interstices between the foam cells there are found other, much smaller, spindle-shaped or stellate cells with round or oval nuclei. These are morphologically indistinguishable from fibroblasts and are usually identified as such. These cells may be quite prominent in the early stages,²⁰ but more often they are not very numerous until the lesions become more advanced. The description by some of the

(e) 66, 147, 148

(f) 4, 100

(g) 45, 70

earlier investigators of smooth muscle cells in the intima seems surely to have been due to a mistake in the identification of the spindle-shaped cells or in the recognition of the limits of the media after the internal elastic lamina had disintegrated. Several authors also mention the occasional occurrence in the intima of small mononuclear cells resembling blood lymphocytes.

Even after the appearance of numerous cells in the intima, the homogeneous intercellular ground substance may remain quite conspicuous, and it appears to increase in abundance. Fine strands of fibroglia and delicate elastic fibrils are also found ramifying between the cells of the intimal thickening.

Opinion varies as to the rôle in the reaction played by the lining endothelial cells. Amitschkow¹ believed that they were entirely passive, and stated that they could always be seen forming a single thin layer of flat cells covering the surface of the thickening in the intima. However, this observation has not always been confirmed. Most authors have found many of the intimal cells covering the lesion somewhat swollen and containing fatty granules, while other cells were thin and flat. But they hesitate to call the swollen cells lining endothelium, although the cells are apparently in contact with the blood stream. This hesitancy is perhaps justified because of the frequent difficulty in seeing any endothelial lining in the normal rabbit's aorta and the ease with which it can be wiped off in handling the gross material.

In frozen sections stained for fat, the aortic lesions are seen to contain large amounts of lipid material. The cytoplasm of the foam cells is packed with innumerable fine granules or droplets which stain with sudan III and which, in unstained sections, show a pale yellow luster. Many of the spindle-shaped cells contain fatty granules scattered in the cytoplasm at either end of the nucleus. The intercellular ground substance contains deposits of finely divided lipid material or it may be densely and uniformly impregnated with lipoids. Crystals of lipid material may be seen in the foam cells as well as in the intercellular ground substance of the intima and, in paraffin sections, fusiform lipid-crystal clefts are frequently seen in both situations.¹ A large proportion of the fatty material is anisotropic. In consideration of this fact and of the other physical properties as well as the staining reactions of the lipid material, it seems probable that the deposited lipoids consist chiefly of cholesterol and its esters.⁴ However, this cannot be stated with certainty since the staining reactions and other criteria commonly employed for the identification of lipoids in histologic sections are subject to certain fallacies, as Kutschera-Aichbergen has shown.²

(h) 4, 12

(i) 45, 100

Chemical determinations of the lipoids contained in the arterial lesions of experimental cholesterol arteriosclerosis have apparently never been made

With the further development of the lesions, the thickness of the intima may reach 126 microns,³ or it may be even greater than that of the underlying media²⁰ The foam cells in the deepest part of the plaque become necrotic, leaving their load of fatty material free in the finely granular debris of their disintegration At the same time fibroblasts come into prominence They proliferate extensively in the deeper layers of the intimal thickening and frequently form a compact fibrous layer just beneath the intimal surface There is a marked increase in fibroglia and elastic fibrils, the latter often showing a connection with the splitting internal elastic lamina A large number of foam cells still persist, scattered singly and in groups throughout the lesion An aggregation of foam cells usually surrounds the collection of free lipid material in the depths of the intimal thickening and adds to its bulk through necrosis The "pool" of free fatty substance which results tends to become calcified, and the calcification tends to involve also the underlying media, which by this time has usually undergone extensive changes

In order to appreciate properly the significance of the deposition of lipoids in the media of the aorta, one must return to the earliest microscopic changes in the vessel and follow the process As has been mentioned, the changes in the media may be the earliest to appear and may remain the most striking So far as I am aware, there is no record in previous literature of lipid deposits in the media preceding those in the intima, although Bailey²⁰ remarked on the extent of the medial lesions in some of his rabbits when the intimal deposits were only beginning However, I have observed in rabbits of my own experimental series⁴⁵ very conspicuous deposits of anisotropic lipid material in the media of the arch before any change could be detected in the overlying intima either grossly or microscopically These deposits were preceded by a peculiar focal necrosis of groups of muscle fibers in the inner third of the media The affected areas were at first quite small, occupying the space between two adjacent elastic laminae and extending for a short distance in the longitudinal direction In such areas there were no intact muscle cells, the spaces containing only a very pale-staining rather cloudy or flocculent ground substance with occasional fragments of cells The smaller areas of necrosis never showed any lipid deposits, but in the larger ones the beginning accumulation of fine granules or droplets of anisotropic lipid became evident The lipid droplets were diffusely distributed through the flocc-

culent ground substance, and in still larger areas lipoids were even more abundant. The fatty material was almost exclusively extracellular and lay free in the anuclear areas of muscle destruction. Occasional small mononuclear cells contained a few tiny droplets of stainable fat in their cytoplasm. However, the abundant accumulations of fat-laden foam cells, so common in the lesions of the intima, were entirely lacking in the media.

The more severe degrees of medial damage in these animals always had associated with them a more or less marked change in the overlying intima, and this appears always to have been the case in the observations of others. With advancement of the medial lesions, there occurs widespread destruction of the muscle and elastic tissue in the inner third of the media followed by a progressive infiltration of lipoids. The individual areas of anisotropic lipid deposit finally coalesce so that the inner medial zone comes to consist only of pale-staining structureless ground substance densely impregnated with lipoids and containing only occasional distorted muscle cells and fragments of elastic fibers scattered through it. The internal elastic lamina resists destruction for a long time, but finally it, too, shows breaks in its continuity. This event marks the first appearance of the large foam cells in the media. They can be seen first near the points of rupture of the internal elastic lamina and seem to be wandering outward from the intimal layer.¹ The internal elastic lamina may eventually undergo complete disintegration, and the inner layer of the media, packed with fat-laden foam cells, becomes almost indistinguishable from the intima. The destruction of the media may advance until only its outer third remains intact. This widespread destruction with weakening of the media is evidently the basis for the formation of the aortic dilatations and aneurysms which may occur in the late stages of the process.⁸⁹

A number of investigators¹ have described the early anuclear areas of fatty infiltration in the media, but the fact of the occurrence of the medial lesions has never been given any prominence. They are frequently given mere mention, and it is tacitly implied that they are only the result of "fatty degeneration" of the media secondary to the intimal lesions. It is true that they are commonly associated with lesions in the overlying intima. However, it is clear that they sometimes occur independently, and that the lipid accumulations are not due to a "fatty degeneration" of the muscle fibers but represent the result of lipid deposition following injury and necrosis of the media. These facts are of considerable importance in the interpretation of the significance of experimental cholesterol arteriosclerosis.

(k) 45, 100

(l) 20, 26, 70, 89, 147, 148, 152, 166, etc

(c) *Changes in Arterial Lesions Following Cessation of Cholesterol Feeding*—The fate of the lesions in the aorta after the feeding of cholesterol is discontinued is a point on which less information is available. However, several investigators^m have reported a few experiments in which the lesions were examined at intervals up to six months after the cessation of cholesterol feeding. Anitschkow¹¹ carried out more extended experiments in which the intervals after the withdrawal of cholesterol from the diet ranged from 101 to 815 days. Similar investigations of the lesions in the coronary arteries of the heart have been reported.ⁿ In all of these experiments, cholesterol feeding was continued long enough for the lesions in the arteries presumably to be well developed. After the cessation of cholesterol administration, the arterial lesions were found to persist to the end of the longest experiments, but their character was somewhat altered. No appreciable changes could be seen in the aortic lesions within a month,¹⁶⁹ but after longer intervals they showed evidences of transition toward a more fibrous type.

The results of these experiments indicate that the fatty materials in the plaques gradually diminish in quantity, a process which seems to be more rapid in the arch of the aorta than in its more distal parts.¹¹ The superficial layers of the thickenings come to be composed chiefly of fibrous connective tissue cells interspersed between abundant collagen and elastic fibrils. Lipoids still persist, however, especially in the deeper parts of the plaques, where numerous anisotropic crystals are found lying in necrotic debris. Calcification in these areas is a frequent occurrence. The fat-containing foam cells diminish in size and number and are found mostly about the collection of free fatty material in the depths of the lesion. Muscle fibers are frequently mentioned as occupying a similar situation. Their presence probably indicates disintegration of the internal elastic lamina with medial involvement, but it is impossible to be certain of this since the condition of the underlying media has not been specifically described. The smallest lesions which remain are composed almost entirely of fine fibrillar connective tissue, but even in these there are found a few fat-containing foam cells or scattered lipid crystals. Hyalinization of the fibrous tissue has never been described as occurring in the intimal thickenings, even at the end of these prolonged experiments.

It would be interesting to know whether or not the earliest minimal lesions would disappear completely after the cessation of cholesterol feeding.

(d) *Mutual Independence of Experimental Cholesterol Arteriosclerosis and Spontaneous Disease of the Arteries of Rabbits*—Before

(m) 80, 131, 152, 169

(n) 81, 184

proceeding farther, it is necessary to consider the possible rôle of spontaneous arterial disease in the development of the arterial lesions described in the foregoing pages. This is especially important in the case of the rabbit, which is the animal that has been used in the great majority of experiments with cholesterol feeding and in which spontaneous disease of the arteries is well known to occur.

Descriptions of spontaneous changes in the arteries of rabbits^o are confined almost exclusively to one type of lesion in which alterations are found chiefly in the media of the aorta. The lesions occur in localized areas in the middle of the medial coat or nearer its inner margin. The muscle cells become necrotic and disappear and the elastic fibers, while remaining intact, lose their undulations and fall together in parallel rows. Calcification in the affected areas is a common end-result. Stainable fat, if present at all, occurs in only the most minute amounts within degenerating muscle cells or in scattered wandering cells, and it is not anisotropic. The intima is affected secondarily, showing inconspicuous alterations in the lining endothelium and some swelling of the sub-endothelial ground substance. The frequency with which this type of spontaneous lesion occurs gives some indication of the susceptibility of the rabbit to arterial injury, but the lesion itself need not be considered further, since it is perfectly clear that it is quite different from the lesions produced by the administration of cholesterol-rich diets. When spontaneous medial lesions are present in a rabbit which has been subjected to cholesterol feeding, they can still be recognized with the greatest ease and distinctly differentiated from the lesions resulting from the feeding.³⁶ I have thoroughly convinced myself of the truth of this statement in my own experiments.

Nuzum and his co-workers¹¹³ recently drew attention to another type of arterial lesion which they encountered in rabbits as a spontaneous development. The lesions which they described are indistinguishable from those which follow the administration of cholesterol-rich diets. In the examination of 190 rabbits, lesions of this type were found in the aortas of 6 of them. All of the animals were between 2 and 3 years old, and most of them had been used in the standardization of insulin or as controls in various experiments. The importance of the observation cannot be denied, but it loses much of its significance in the face of the evidence accumulated by a number of other investigators.

So far as I am aware, the only report of a similar finding is that of Ophuls,¹¹⁶ who found lesions of the type in question in the aorta of 1 rabbit. The animal had just been received in the laboratory and its age was unknown, but it was described as a large, healthy, well developed, female rabbit. Ophuls thought the observation sufficiently

(o) 23, 36, 43, 61, 87, 95, 96, 101, 113, 118, 171

unique to warrant a separate publication. On the other hand, similar lesions have never been encountered among the several thousands of rabbits examined by other investigators¹ in search of spontaneous arterial disease. Failure to find the lesions could hardly be the result of failure to examine the arteries microscopically, for in most instances microscopic examinations were made. In view of these facts, it seems hardly credible that spontaneous arterial lesions of this type, heretofore so excessively rare, should suddenly crop out in a relatively small series of 190 animals without some definite cause. It is, of course, impossible to know exactly what the cause was, but it seems that the use of some of the animals for apparently innocent experimental purposes may not have been entirely without effect. Dietary factors acting over a long period of time may have been responsible. This is possible since the rabbits were all old ones, and the suggestion seems all the more plausible since it is known that during the prolonged administration of diets containing very small quantities of cholesterol, lesions of the type in question develop in the aorta.⁷ The same explanation may well apply in the case of the rabbit described by Ophuls, the previous diet of which was entirely unknown. In any event, confirmatory reports from other laboratories in which the routine diets for rabbits is different would be requisite to a firm conviction regarding the strict spontaneity of the aortic lesions under discussion.

Whatever may be one's opinion concerning the cause of these "spontaneous" changes, it still seems certain that the fatty arterial lesions observed in the numerous experiments on rabbits were produced by the experimental procedures employed, and this for three reasons. 1. In no series of experiments have lesions of the type in question been found in control animals. 2. Barring accidents, the arterial lesions can be produced without fail in young rabbits by the use of sufficient doses of cholesterol administered in a suitable way. 3. Very young rabbits are just as susceptible to the effects of cholesterol feeding as adult animals. This fact is illustrated in the experiments of Starokadomsky,¹⁴⁷ who was able to produce typical fatty lesions of the aorta in all of 6 rabbits which at the end of the experiment were only 80 days old. For these reasons it is concluded that the arterial lesions of experimental cholesterol arteriosclerosis in rabbits may safely be considered as one of the results of the feeding of cholesterol-rich diets.

V EFFECTS OF CHOLESTEROL-RICH DIETS ON THE ARTERIES OF ANIMALS OTHER THAN RABBITS

The method of cholesterol feeding has now been applied to a variety of experimental animals in attempts to produce fatty lesions in the arteries, but for the most part without success. It seems probable that

experimental arteriosclerosis can be produced in guinea-pigs by cholesterol feeding but only with considerably greater difficulty than in rabbits. Fatty lesions in the aorta and sometimes in other arteries have been observed in pigeons, chickens, a parrot, mice, rats and goats following prolonged periods of cholesterol feeding. All of the experiments on the latter animals, however, are open to serious objections. The possibility that the arterial lesions had arisen spontaneously has been almost completely ignored, and in many instances control observations on the arteries of untreated animals are entirely lacking. Nevertheless, these experiments are frequently cited as evidence that experimental cholesterol arteriosclerosis can be produced in a wide variety of animals. In certain other animals the failure of cholesterol feeding to affect the arteries is admitted by every one, all attempts to produce arterial lesions in cats, dogs, foxes and monkeys by feeding cholesterol or cholesterol-rich diets have been uniformly without success.

The experiments on the various animals mentioned will be outlined as briefly as possible. However, some of the experiments must be described in greater detail than they deserve, merely to demonstrate their inadequacy as a basis for the statements concerning them which constantly recur in the literature.

(a) *Birds*—The literature dealing with the results of cholesterol feeding in birds is extremely unsatisfactory though frequently quoted. The experiments are few in number and poorly controlled or not controlled at all, so that the results are far from convincing. So far as I can determine, there are recorded in the literature experiments on 7 pigeons, 20 chickens and 1 parrot. These experiments will be considered in order.

Pigeons. Anitschkow¹² has referred to three investigators as authority for his statement that lipid infiltration of the arterial walls can be produced in pigeons by feeding them with egg yolks, hydrous wool fat or cholesterol. Of these investigators, only Yamaguchi¹⁸⁷ reported any experiments on pigeons, his pigeons were fed with egg yolks or hydrous wool fat. The experiments were described in Japanese in 1922, and the only available source of information concerning them (and the source to which the European investigators refer) is Kawamura's monograph,⁷⁰ in which Yamaguchi's report of his experiments with pigeons and chickens is quoted at length. Apparently Yamaguchi fed 4 pigeons with 1 or 2 egg yolks daily for periods of from 562 to 603 days. Three other pigeons were fed quantities of hydrous wool fat for even longer periods. It is not clear what lesions were found in the arteries at the termination of the experiments, for Kawamura has described together the results obtained by Yamaguchi in both pigeons and chickens. Some parts of the description refer specifically to chickens, some parts refer to pigeons, but other parts may refer to either or both. However, this much seems

to be clear. The 4 pigeons fed with egg yolks showed gross "atherosclerosis" of the aorta, in 1 of these there was "a general thickening of the first part of the aorta." The 3 pigeons fed with hydrous wool fat showed no gross lesions but evidently some thickening of the intima was seen microscopically. In describing the microscopic changes in the arteries, Kawamura failed to distinguish between the lesions found in pigeons and those in chickens, but one gathers that the pigeons' aortas probably showed some deposits of lipoids in the intimal thickenings and in the inner layers of the media.

Whatever may have been the exact nature of the lesions observed in the arteries of the 7 pigeons, there is absolutely no proof that the lesions were not spontaneous nor is there any proof that they had developed as the result of feeding the pigeons egg yolks or hydrous wool fat. No control animals were mentioned, and Kawamura himself seemed to doubt that the cholesterol in the diet had produced the changes in the arteries. In another part of Kawamura's monograph are recorded Yamaguchi's observations on the distribution of cholesterol in the organs of a variety of species of birds including pigeons, all presumably normal, but there is no mention of the condition of the arteries in the pigeons or in any of the other birds excepting chickens. Spontaneous arteriosclerosis, however, does occur in pigeons, as described by Fox.⁴⁸

Although cholesterol-rich diets may be capable of producing lesions in the arteries of pigeons, it is difficult to understand how any one can regard this possibility as an established fact on the basis of the nebulous information outlined in the preceding paragraphs. One can conclude only that the feeding of cholesterol-rich diets has not been shown to have any effect whatever on the arteries of pigeons.

Chickens. In conjunction with his experiments on pigeons, Yamaguchi¹⁸⁷ also experimented on 11 chickens, which he fed daily with egg yolks or hydrous wool fat for periods up to 807 days. Two of the 5 chickens which had been fed egg yolks showed "atherosclerosis" of the aorta, and in 1 of the 2 there were distinct changes in the intima of the large branches of the aorta. The arteries of the 6 chickens which had been fed with hydrous wool fat showed no gross lesions. In commenting on Yamaguchi's experiments with pigeons, I have pointed out the difficulty of obtaining from Kawamura's résumé of the experiments any exact information concerning the lesions in the arteries. The same difficulties are encountered in the case of the experiments on chickens. Again no control animals were mentioned by Kawamura, although he remarked in another part of his monograph that Yamaguchi had observed arteriosclerosis in apparently normal chickens. Uchiyama,¹⁶⁰ who presumably had access to Yamaguchi's Japanese papers, stated that the latter had found spontaneous "atherosclerosis" of the

aorta in about 75 per cent of all the untreated chickens examined and that he had not observed any increase in the arterial changes in chickens fed egg yolks

The frequent occurrence of spontaneous arteriosclerosis in chickens is well recognized. The spontaneous lesions in the arteries have been described in detail by Uchiyama,¹⁶⁰ who found that they were almost always present in the aorta by the end of the second year of life. The lesions consisted of localized accumulations of lipoids in the intima and inner layers of the media with fibrous proliferation in the former and sometimes with the formation of atheromas. Necrosis and calcification of the media were found in more diffuse distribution, especially in the distal half of the aorta, which in chickens has the structure of a muscular artery. Uchiyama also carried out feeding experiments on 9 chickens which were fed daily 0.3 Gm of cholesterol in oil for periods of from 12 to 420 days. In some of these chickens he found arterial lesions of exactly the same character and distribution as those occurring spontaneously in the control animals, but he believed that the intimal changes in the chickens of the experimental group were more advanced. Confirmation of this opinion by clearcut and carefully controlled experiments will be necessary to any firm conviction regarding the ability of cholesterol feeding to affect the arteries of chickens.

Parrots. Wolkoff¹⁸³ has described the lesions found in the arteries of a 40 year old parrot which had been fed egg yolks almost daily during the last 3 years of life. This apparently is the parrot referred to by Amitschkow.⁹ Both Wolkoff and Amitschkow seemed to think that the feeding of egg yolks had had some bearing on the production of the arterial lesions, but this can be nothing more than an opinion since no control animals were examined. Identical spontaneous arteriosclerotic changes have been found by a number of different investigators^q in parrots of comparable age or younger given diets containing no foods especially rich in cholesterol. There is no reason, therefore, to believe that the lesions in the arteries of the parrot described by Wolkoff were in any way dependent on the feeding of egg yolks, and it seems altogether likely that they were of spontaneous origin.

(b) *Mammals*—Guinea-Pigs. As early as 1913 Chalataw³⁰ had tried the effect of lipid diets on several guinea-pigs. Anisotropic lipid deposits were found in 1 animal, chiefly in the liver and spleen, but no mention was made of any changes in the arteries. However, in 1915 Bailey¹⁹ succeeded in producing fatty lesions in the aortas of guinea-pigs by feeding from 0.1 to 0.5 Gm of cholesterol in oil daily for periods up to 72 days. No alterations were visible to the naked eye, but microscopically he found "small patches of fatty infiltration in the intima

(q) 22, 48, 60, 111, 117

and upper media. The characteristic proliferation and subsequent degeneration seen in the rabbit were entirely lacking." He felt that the feeding period was too short to exclude the possibility that proliferative changes might occur.

Anitschkow⁶ repeated the experiment some years later on 7 guinea-pigs which he fed from $\frac{1}{3}$ to 1 egg yolk daily. The longest experiment lasted 183 days. In the 3 animals which were fed for more than 62 days, he was able to make out minimal gross changes in the form of isolated tiny yellowish streaks in the first part of the arch of the aorta. Microscopically the lesions consisted of a diffuse infiltration of lipid material in the subendothelial layer of the intima and in the inner layers of the media. Drawings show clearly that the fatty deposits in the media were located in anuclear areas from which the muscle cells had disappeared. The more advanced lesions showed cellular accumulations in the intima consisting of cells like small blood lymphocytes and of large fat-laden foam cells. The internal elastic lamina was sometimes split and connected with fine elastic fibrils in the thickened intimal layer. In some cases it showed breaks near which cells were found in the media similar to those seen in the intima.

The briefer reports of several other investigators⁷ lend corroboration to these observations.

Unfortunately, no control animals appear to have been studied in connection with any of these experiments, and this fact introduces some doubt as to the status of the arterial lesions described. I have been unable to find much information concerning the occurrence of spontaneous disease of the arteries of guinea-pigs. Krause⁷⁸ and Lowenthal⁹⁴ did not mention guinea-pigs in their reviews dealing with spontaneous arterial diseases in animals. However, Weinberg¹⁷¹ stated that he had examined the aortas of 236 guinea-pigs without encountering arterial lesions of any kind. Saphir¹⁸⁰ also reported the absence of lesions in the aortas of 30 apparently normal guinea-pigs. In view of these completely negative observations in normal animals and in consideration of the fact that arterial lesions were found in a fairly large proportion of the experimental animals, it seems that the changes in the arteries of guinea-pigs attributed to cholesterol feeding may be tentatively accepted.

Mice. The first reports of fatty changes in the arteries of cholesterol-fed mice were made by Lowenthal⁸ in 1925. He experimented with white mice, which he fed daily 0.015 Gm of cholesterol in oil. In addition to being fed cholesterol, some of the mice were given egg-white and others were castrated. Only 1 of the 4 mice which received no treatment other than cholesterol feeding presented any fatty deposit in the arteries. It had been fed for about $4\frac{1}{2}$ months, and at the end

(*r*) 70, 135, 156

(*s*) 92, 93

of that time it showed a single microscopic area of fat deposit just above the aortic valve ring. The subendothelial layer of the intima contained fatty granules, and a few small cells also contained lipid material. Among the 22 mice fed cholesterol and egg-white and in the emasculated group, 12 animals had microscopic fatty lesions in the aorta, characterized by an accumulation of finely divided lipid material in the subendothelial layer, part of which was contained in spindle-shaped cells. Some of the aortas also showed an infiltration of the inner layers of the media with fatty granules.

Yuasa¹⁸⁸ fed mice daily 0.03 Gm of cholesterol in 0.2 Gm of lard for from 80 to 158 days. Of the 14 mice, 6 showed fatty lesions in the aorta, the minimum time for their development being 126 days. The changes in the aorta were not described in great detail. They consisted essentially of an accumulation of anisotropic lipid droplets in the intima and neighboring media with some cellular proliferation in the former.

Wolkoff¹⁸⁹ found similar microscopic aortic lesions in 3 of 11 mice which had been fed cholesterol-rich diets. In one animal, feeding had been in progress for 182 days, and in the other 2, for 425 days.

Although Wolkoff reported 6 control animals in which there were no aortic lesions, nevertheless, mice appear to be subject to the development of spontaneous arterial changes which may be identical with those found after cholesterol feeding. Lowenthal⁹⁴ found such microscopic changes in 9 mice among about 85 which he examined. In his experiments with cholesterol feeding⁹³ he found arterial lesions in 13 of 26 mice, but 12 of the 13 had been castrated or fed egg-white in addition to being fed cholesterol and, moreover, 3 of the 6 control animals also showed arterial lesions. Yuasa apparently made no observations on the arteries of control animals.

On the basis of such evidence, one seems hardly justified in concluding that cholesterol feeding alone is capable of producing arterial lesions in mice. Indeed, lack of adequate controls, together with the fact that similar arterial lesions occur not infrequently in untreated animals, renders these experiments practically worthless for the purposes of this paper.

Rats. In his earlier experiments Chalutow³¹ was unable to produce deposits of anisotropic fat in the organs of white rats without the addition of phosphorus or fatty acids to the cholesterol feeding. Although he then found abundant deposits of anisotropic lipids in the spleen and isotropic fat in the liver and suprarenal glands, he found no changes in the arteries. In a later series of 24 experiments³² he obtained similar results. No arterial changes were produced even by heavy and prolonged feeding of cholesterol except in 1 animal. This rat had received a total of about 35 Gm of cholesterol in oil within 4½ months. Fatty acids and phosphorus were also administered in varying quantities.

The aorta of this animal showed intimal thickenings in which were found deposits of finely divided anisotropic fat, partly contained within large phagocytic cells and partly dispersed through an abundant homogeneous intercellular material

In Kon's experiments with rats fed various lipid diets⁷⁰ arterial changes were completely lacking

In consideration of the great rarity of arterial lesions in the experiments of previous investigators, Yuasa's results¹⁸⁸ seem rather surprising His rats were given a daily dose of 0.1 Gm of cholesterol in from 1 to 2 Gm of lard Five of the 9 rats showed slight deposits of anisotropic fat in their aortas, the earliest change being found after 96 days of feeding The lesions consisted of an accumulation of numerous droplets or granules of anisotropic lipoids in the intima and inner layers of the media

In Yuasa's account of his experiments with rats no reference was made to any observations on the arteries of control animals Chalataw described 2 apparently normal rats as controls for his 24 experiments with cholesterol feeding, etc, but unfortunately he failed to mention the condition of the arteries of the control animals I have been unable to find any independent information concerning the occurrence or frequency of spontaneous arteriosclerosis in rats In the complete lack of such information from any source, and in view of the rarity of arterial lesions in rats following cholesterol feeding, one is forced to exclude these experiments from further consideration

Goats So far as I am aware, Chalataw's observations on goats fed cholesterol-rich diets³³ are the only ones recorded in the literature In his experiments, 3 goats were fed from 5 to 15 egg yolks daily for from 2½ to 5 months The goat which had been fed for the longest time, which had received the greatest total number of egg yolks and in which the blood cholesterol reached the highest level, showed no lesions in the arteries at the termination of the experiment In 1 of the other goats, there was found a single small fleck of fat in the intima of the arch of the aorta The aorta of the third goat showed many small flecks of fat in the intima, which were grouped mostly about the mouths of branching vessels No control observations on the arteries of untreated goats were recorded by Chalataw, so that there is really no evidence to show that the administration of egg yolks was responsible for the development of the arterial lesions

The occurrence of spontaneous arteriosclerosis in goats is well recognized⁷⁸ It usually takes the form of a calcification of the media of the aorta, but the possibility that lipid deposits in the intima may occur as a spontaneous development has not been excluded, for this possibility has apparently not been investigated With these facts in view, it is obvious that Chalataw's experiments, though frequently cited, cannot

be accepted as a demonstration of the ability of cholesterol-rich diets to produce lesions in the arteries of goats

Cats Yuasa¹⁸⁸ carried out experiments on 8 cats which he fed daily with a mixture containing meat extract, lard and 1 Gm of cholesterol. The longest experiment lasted 120 days, but in none of the animals did he find any alterations in the arteries.

The experiments of Cilio³⁵ were more protracted. He fed 5 cats cholesterol and cholesterol-rich substances for long periods of time. The longest experiment lasted 394 days, during which time the animal was given over 300 Gm of cholesterol and nearly 40 Kg of brain tissue. However, no arterial changes were found in this cat or in any of the others.

Dogs The experimental results of Adler¹ have been cited as evidence that fatty changes in the arteries may follow cholesterol feeding in dogs. However, these results cannot be considered as significant, for Adler himself in a subsequent publication² stated that he had observed, in the interval, similar fatty changes in the arteries of normal untreated dogs. He felt that no importance could be attached to his earlier observations of fatty striae in the aortas of dogs which had been fed cholesterol.

Tsunoda and Umehara¹⁵⁷ briefly reported their failure to produce any changes in the arteries of dogs by feeding them for from 1 to 2 years on large quantities of brain tissue. In Kawamura's experiments on dogs⁷⁰ the arteries were found to be normal after the prolonged feeding of cholesterol or egg yolks.

Pfleiderer¹²¹ experimented with 3 dogs, each of which was fed 426 Gm of cholesterol in oil in the course of 496 days. The dogs were also given considerable quantities of irradiated ergosterol, but no lesions were to be found in their arteries at the end of the experiments.

Anitschkow⁹ fed a 6 month old dog from 4 to 5 Gm of cholesterol in oil daily for 14 months. In addition, he produced marked stenosis of the common bile duct by means of ligatures and added to the cholesterol intake by administering bile from another dog with a biliary fistula. He also removed both ovaries and the spleen. By these combined procedures, the blood cholesterol content was raised to about twice its normal value. Nevertheless, no fatty deposits could be demonstrated in the aorta or in the valves of the heart.

Foxes Yuasa¹⁸⁸ fed 2 young foxes raw meat and lard for 3 months. In addition, 1 of the foxes was given a daily dose of 1 Gm of cholesterol dissolved in the ration of lard. At the end of the experiment, however, no lesions were found in the arteries of either fox.

Monkeys The only recorded experiments on monkeys are those of Kawamura,⁷⁰ which seem to be of the utmost importance. He used

3 monkeys of the species *Macacus fuscatus*. One was fed 2 egg yolks daily for 150 days. The other 2 were fed quantities of hydrous wool fat. One of these received a total of 1,350 Gm of hydrous wool fat in 301 days, and the other, 1,650 Gm of the same substance in 303 days. At the termination of the experiments, no changes in the aorta could be distinguished in any of the monkeys. The liver, spleen and bone marrow contained only insignificant amounts of anisotropic lipoids or else none at all, while the suprarenal glands contained about the same amounts of lipoids as were found in normal control animals.

From the data which have been presented in the foregoing pages, the following conclusions may be drawn. It seems highly probable that experimental cholesterol arteriosclerosis can be produced in guinea-pigs by means of prolonged feeding with cholesterol-rich diets. There is no satisfactory experimental evidence to substantiate the current idea that cholesterol-rich diets can produce lesions in the arteries of pigeons, chickens, parrots, mice, rats or goats. All experiments recorded up to the present time indicate the inability of intensive and prolonged cholesterol feeding to affect in any way the arteries of cats, dogs, foxes or monkeys.

VI INFLUENCE OF VARIOUS FACTORS ON THE DEVELOPMENT OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS

Under this heading, only the experiments on rabbits and guinea-pigs need be considered, for these two species are the only ones in which unequivocal cholesterol arteriosclerosis has been produced experimentally. Of the two species, rabbits have been used much more commonly in experiments with cholesterol feeding, and consequently most of the information available for analysis is concerned with experimental cholesterol arteriosclerosis in rabbits. However, the meager information which has arisen from experiments with guinea-pigs will also be presented. In addition, references will be made to experimental data obtained from the study of other animals whenever such information appears to be pertinent to the subject or of interest for purposes of comparison.

(a) *Cholesterol in the Diet* — There is little remaining doubt that the feeding of cholesterol is one essential procedure in the experimental production of the arterial lesions in rabbits which have been described in a previous section of this paper (section IV). The lesions in the arteries produced by feeding pure cholesterol not dissolved in oil are indistinguishable from those which follow the administration of cholesterol in oil or of diets containing cholesterol-rich foods such as egg yolks. Oils which have been used as solvents and other neutral fats and fatty acids do not have similar effects in control experiments. Wesselkin's experi-

ments[†] indicate that lecithin is not responsible." Indeed, arterial lesions of the type in question have been produced in rabbits only when the administration of cholesterol or of substances containing considerable amounts of cholesterol was part of the experimental procedure. This is the justification for the application of the term "experimental cholesterol arteriosclerosis" to the arterial lesions under consideration. Of course, the possibility still remains that some potent contaminant of supposedly pure cholesterol is also an essential causative or contributory factor, and there are good reasons for believing that lipoids are deposited in the arteries only after the latter have been injured by the action of an unknown damaging agent. Nevertheless, it seems certain that cholesterol administration is one etiologic factor in the production of the experimental arterial lesions in rabbits, that it is the only factor remains open to question.

In guinea-pigs, the effect of adding solid cholesterol to the diet has not been tried. However, arterial lesions have been produced by feeding cholesterol dissolved in oil,¹⁹ and in all other successful experiments with guinea-pigs cholesterol-rich diets were employed. Thus, it seems altogether likely that the etiologic rôle of cholesterol feeding in the development of the fatty arterial lesions is the same in guinea-pigs as in rabbits.

In connection with the feeding of cholesterol the analysis can be carried a little farther. It is obvious that a considerable total quantity of cholesterol must be administered (at least from 10 to 20 Gm. in the rabbit) and that a certain length of time must elapse before any alterations can be detected in the arteries. In general, it is true that large daily doses of cholesterol produce arterial changes more rapidly than do small doses, but the rapidity of the development of the lesions is dependent also on the way in which cholesterol is administered. However, even when cholesterol is given in the most favorable form and in large daily doses to rabbits, microscopic changes in the arteries appear only after from 25 to 30 days of feeding. About twice that length of time is usually necessary for the production of gross lesions. The importance of the factor of time is illustrated by an experiment of McMeans and Klotz.¹⁰⁰ They gave a rabbit daily feedings of cholesterol

(†) 178, 179

(u) Kanocz and Laszlo⁶⁸ claimed that lecithin has an effect antagonistic to that of cholesterol. The experiments which they offered as a demonstration of this effect were carried out on only 4 rabbits, and the results were ruined by the chance occurrence of calcified spontaneous lesions of the aorta in the 2 animals which had been fed cholesterol without the addition of lecithin. The true character of these arterial lesions is perfectly obvious from the illustrations in their paper, but the authors apparently failed to recognize the spontaneous nature of the calcified lesions in the media, and cholesterol feeding was held responsible. The conclusions drawn on the basis of these experiments cannot be accepted without verification.

in the form of an emulsion with sodium oleate, a preparation which they found to be even more favorable to the production of arterial lesions than solutions of cholesterol in oil. The experiment was continued for 19 days, and although the quantity of cholesterol administered reached the large total of 75 Gm., no gross or microscopic fatty deposits could be found in the arteries. Thus it is clear that the simple administration of cholesterol even in a favorable form does not begin to show its effects on the arteries of rabbits until at least 20 days have passed. So far as I am aware, no trace of lipid deposits in the arteries of rabbits has ever been observed within a time less than 20 days from the commencement of cholesterol feeding. Even the production of lipid deposits in less than 25 days is most exceptional, being reported only by Zinserling¹⁸⁹. This period represents the time necessary when no procedure other than cholesterol administration is employed, and it serves as a useful criterion in estimating the effects of other procedures which may be combined with the administration of cholesterol. Beyond this minimum limit, the factor of time gradually diminishes in importance, and the first appearance or the severity of the lesions comes to depend rather on the total quantity of cholesterol and on the way in which it is given.

No matter in what form cholesterol is fed, it seems to be capable of producing experimental arteriosclerosis in rabbits eventually, if its quantity is sufficient. However, some forms of administration bring results more quickly than others even when the daily dose of cholesterol is the same. The most outstanding difference is to be noted between the feeding of cholesterol in dry powdered form and its administration in oily solutions or in natural emulsions (e. g., egg yolks or hydrous wool fat). To illustrate this point the experience of Wacker and Hueck¹⁶⁶ may be quoted again. They found arterial lesions in their rabbits only after a period of 5 months when about 175 Gm. of powdered cholesterol had been fed. Knack⁷⁴ fed a rabbit 382.5 Gm. of solid cholesterol in 85 days and failed to produce any recognizable lesions in the arteries. This is in marked contrast with the earlier appearance of arterial lesions produced by the feeding of much smaller quantities of cholesterol dissolved in oil or contained in egg yolks. Knack⁷⁴ and Sternberg¹⁵¹ pointed out this difference years ago, and their observation has since been reiterated by others, but some investigators still seem to be unaware that the feeding of solid cholesterol is not comparable with its administration in oily solutions or in association with other lipids as it occurs in the cholesterol-rich foods from natural sources. Failure to appreciate this fact has led to some anomalous conclusions.

The exact reason for this difference is not entirely clear, but the most obvious and simple explanation is that solid cholesterol is not so readily absorbed from the alimentary tract as is cholesterol in oily solutions or in fatty emulsions. The fats not only aid in absorption through their

action as solvents but are themselves absorbed and appear in the blood stream with the cholesterol. It is believed that they serve to hold the cholesterol there by combining with it, partly by the formation of esters and partly in a physico-chemical way.¹

It is evident that the kind of experimental animal used is a factor of importance. Experimental cholesterol arteriosclerosis can be produced in rabbits with relative ease, but it is a matter of greater difficulty in guinea-pigs.² On the other hand, even the most persistent efforts to produce arterial changes in cats, dogs or monkeys by means of cholesterol feeding have met with complete failure. These differences clearly depend, at least in part, on the facility with which the various animals handle exogenous cholesterol. The degree of susceptibility of their arteries to damage probably also plays a rôle.

(b) *Hypercholesteremia*—Interest in the chemistry of the blood of cholesterol-fed animals has centered naturally in the fluctuations of the blood cholesterol and lipoids, and unfortunately other chemical problems have been almost completely neglected. However, the results of studies on the lipoids of the blood are of considerable interest and are of great importance to the purpose of this paper because of the emphasis which has been laid on hypercholesteremia as an etiologic factor both in experimental and in human arteriosclerosis.

The effect of cholesterol feeding on the cholesterol content of the blood varies in different species of animals. Certain carnivores seem to be adequately equipped for the disposal of exogenous cholesterol through utilization, storage or excretion. Thus, dogs respond to single doses of cholesterol dissolved in oil with only a moderate and transient increase in the blood cholesterol content,³ an increase which is often no greater than that which may occur when the same amount of oil is fed without cholesterol.⁴ The continued feeding of cholesterol seems likewise to be incapable of producing any very striking sustained rise in the blood cholesterol level. Grigaut and L'Huillier⁵⁶ found a moderate increase in the cholesterol content of the blood of 2 dogs which were receiving daily feedings of cholesterol and butter, but others have observed no significant hypercholesteremia under similar conditions.² The few investigations of this nature which have been carried out in cats¹ have given results similar to those obtained in dogs. In rabbits, on the contrary, the mechanism for handling exogenous cholesterol and especially for its excretion is notably deficient, so that a quite enormous increase in the cholesterol content of the blood can be

(v) 74, 161, 164

(w) 19, 70

(r) 14, 21, 86, 123, 144

(v) 14, 86

(s) 144, 172, 177

(a) 53, 177

produced by the feeding of cholesterol^b or of egg yolks⁶⁷ Similar results have been obtained in guinea-pigs¹⁷⁷

In rabbits, a course of cholesterol feeding such as is commonly employed for the production of arterial lesions usually results in a very great increase in the blood cholesterol content. Values of from 500 to 1,500 mg per hundred cubic centimeters are not infrequently reported,^c and Rohrschneider¹²⁶ stated that he had found maximum values ranging from 25 to 50 times the normal as a regular occurrence in rabbits fed for long periods with cholesterol dissolved in oil. The highest figure which he recorded is 2,584 mg per hundred cubic centimeters. Such high levels, however, are reached only after cholesterol feeding has been continued for some time. With daily feedings of 0.5 Gm of cholesterol in oil, a few days elapse before the blood cholesterol begins to rise significantly, then it increases rapidly so that at the end of 3 weeks it has risen to 3 or 4 times its original value. The rise is slightly irregular but continuous, and the high maxima mentioned are found after from 3 to 5 months of feeding. The blood cholesterol then drops gradually in spite of continued feeding but does not return nearly to normal.^d All of this concerns the total cholesterol content of the blood. Other investigations have shown that the greater part of the increase is due to a rise in cholesterol esters, while the augmentation of free cholesterol is not so striking.^e This is the case even when pure cholesterol without oil is added to the ordinary diet.¹⁶⁷ In addition to the hypercholesteremia there is a parallel increase in neutral fats and in other lipoids so that a severe lipemia results.^f This shows itself in a milky clouding of the plasma which usually appears within 45 days after the commencement of feeding,¹⁵⁴ but there may be a considerable rise in the total lipoids before distinct clouding becomes visible.⁶²

Although it is probable that hypercholesteremia of extreme degree commonly exists in the rabbit during the course of the usual cholesterol feeding experiment in which arterial lesions are produced, it does not necessarily follow that the hypercholesteremia is responsible for the development of the changes in the arteries. The same might be said of the great increase of total lipoids in the blood. Consequently it is important to know whether experimental cholesterol arteriosclerosis can develop without any increase in the blood cholesterol above its normal limit. It seems doubtful that this is possible but it is clear that excessive hypercholesteremia is not necessary. The lesions in the

(b) 38, 49, 126, 137, 154, 155, 161, 167

(c) 126, 154, 155, 167

(d) 126, 137, 154

(e) 62, 161, 167

(f) 62, 116a, 161

arteries may occur in the presence of a moderate or even a slight increase in the cholesterol content of the blood, as is shown by the following examples

Prolonged experiments were carried out by Amitschkow⁷ on several rabbits which he fed very small quantities of egg yolk in milk. During the experiments a number of chemical examinations of the blood revealed only a slight increase in cholesterol content. At the end of the feeding period he found definite fatty lesions in the aorta. In the experiments of Clarkson and Newburgh¹⁶ and of Deicke¹⁸ certain rabbits, unlike the majority, failed to show more than a slight hypercholesteremia following cholesterol feeding, yet typical fatty lesions were found in their arteries. Certain other rabbits showed a well marked increase of the blood cholesterol, but no arterial lesions were found in them at the end of the experiments. These results led the latter investigators to question the view that the development of the arterial changes is dependent on hypercholesteremia. However, it has never been satisfactorily demonstrated that experimental cholesterol arteriosclerosis can develop in the absence of augmentation of the cholesterol content of the blood. It seems highly probable, and it will be assumed hereafter in this paper, that hypercholesteremia of some degree is an essential factor in the development of experimental cholesterol arteriosclerosis in rabbits.

Now, even if this conclusion is accepted as true, the possibility still remains that hypercholesteremia is not the only essential factor in the development of the arterial lesions of experimental cholesterol arteriosclerosis. It is possible that hypercholesteremia is quite impotent to affect the arteries without the concomitant action of other influences. Indeed evidence is available to suggest that this is actually the case. Several investigators⁸ have recorded observations which show clearly that marked hypercholesteremia may develop during prolonged cholesterol feeding experiments without producing any changes in the arteries within the experimental period, which was greater than 3 months in a number of instances. This point, though not frequently remarked on, is emphasized by Clarkson and Newburgh¹⁶ and by Deicke¹⁸. If the experiments had been continued for a longer time, arterial lesions probably would have appeared eventually, but the fact remains that at the termination of the experiments marked hypercholesteremia was present and probably had been present for a considerable period of time and yet no lesions whatever were found in the arteries. This suggests that hypercholesteremia of itself is not solely responsible for the development of experimental cholesterol arteriosclerosis in rabbits. Some additional factor is probably necessary.

In brief, it seems probable that hypercholesteremia can exist without the development of experimental cholesterol arteriosclerosis, but the latter cannot develop without the existence of hypercholesteremia

(c) *Protein in the Diet*—There is a general impression that the feeding of protein, especially animal protein, with cholesterol enhances the effect of the latter in producing arterial lesions¹¹ This idea was based originally on calculations and comparisons of the cholesterol and protein contents of diets used by different investigators to produce arterial lesions in rabbits Although the impression may be correct, there is still hardly any direct experimental evidence to support it Lowenthal's experiments on mice⁹³ are usually quoted in this connection but, as I have pointed out, there are certain objections to these and other experiments on mice which render them far from convincing

Newburgh and Clarkson¹¹⁰ produced fatty lesions in the arteries of rabbits by feeding them diets which contained a rather large proportion of meat, and they concluded that the protein was responsible In a subsequent publication³⁶ they reported that the feeding of the meat diet resulted in an increase in blood cholesterol, but that the amount of cholesterol contained in the diet, when fed alone, was insufficient to cause arterial lesions or even to raise the blood cholesterol level They thought, therefore, that the elevation of the blood cholesterol level in the rabbits on the meat diet must have been due to a metabolic disturbance produced by the excess of protein, and that it was not related to the cholesterol content of the meat Unfortunately, these conclusions are invalidated by the fact that these investigators in their experiments with the feeding of pure cholesterol administered it in the form of a dry powder so that it probably was not absorbed nearly so completely as was its equivalent in the meat diet Other lipoids were undoubtedly present in the latter to facilitate the absorption of cholesterol The two series of experiments are therefore hardly comparable It is only fair to say, however, that the daily dose of cholesterol calculated to be equivalent to that contained in the meat diet was so small that one cannot feel certain that it would have produced arterial lesions by itself even if it had been administered in a favorable form Nevertheless, the experiments as presented do not provide an unassailable demonstration of the ability of protein to aggravate the effects of cholesterol feeding, and they certainly do not prove that the protein in the meat diet was solely responsible for the development of the lesions in the arteries The direct experiment of feeding a diet rich in proteins and free from cholesterol was not carried out

In connection with the effect of protein administration, Deicke's experiments³⁸ are of interest, but they are no more than suggestive He studied the fluctuations of the blood cholesterol of rabbits following

intravenous injections of colloidal solutions of cholesterol in water. He found the greatest elevations of blood cholesterol following injections of a sol which contained a protein or protein-like substance in addition to cholesterol. He believed that the protein aided in holding the cholesterol in the blood stream. He was unable to produce any vascular lesions by means of parenteral injections, but he used the information gained to explain the results of his feeding experiments in which he had found more pronounced alterations in the arteries following the administration of liver diets than after feeding pure cholesterol in oil.

No conclusions can be drawn from the evidence at hand as to the effect of protein in the diet. The idea that the feeding of protein in conjunction with cholesterol promotes the development of experimental cholesterol arteriosclerosis remains to be substantiated by clearcut experimental evidence. I know of no data to indicate that the administration of sterol-free protein diets can produce arterial lesions similar to those of experimental cholesterol arteriosclerosis.

(d) *Blood Pressure*—The question of the influence of possible fluctuations in the blood pressure of rabbits during cholesterol feeding experiments was first raised by van Leersum⁸⁵ and Fahr⁴⁷ in 1912. Later it was taken up by Schmidtman,¹ who reported a moderate rise of the blood pressure in some of her rabbits during the feeding of diets containing liver. She concluded that the cholesterol content of the liver diet was responsible. Deicke³⁸ and Westphal¹⁸⁰ obtained similar results in rabbits fed with pure cholesterol. However, in none of these series of experiments did the rise in blood pressure occur in all of the rabbits, and it appears doubtful that the fluctuations observed were really outside normal limits.¹ On the other hand, several investigators¹ have reported that observations of the blood pressure at frequent intervals revealed normal values during cholesterol feeding experiments in which severe aortic lesions were produced. Thollde¹⁵⁴ used Schmidtman's method for the determination of blood pressure, and although he produced well marked changes in the arteries of his rabbits by feeding them cholesterol in oil, their blood pressures remained within physiologic limits throughout the experiments. He was also able to show that the repeated passage of a stomach tube, which had been suggested as an additional cause of increase in blood pressure, is without perceptible effect. One possible mechanism by which a rise of blood pressure might conceivably be produced is excluded by the work of Okuneff,¹¹⁴ who showed that the function of the depressor nerves leading from the aortic arch is not

(i) 132, 133

(j) Compare 41, 42, 98

(k) 42, 143, 154, 155

abolished or in any way disturbed by the presence of well developed lesions in the aorta

It is a safe conclusion that a rise of blood pressure is not necessary for the development of experimental cholesterol arteriosclerosis in rabbits It is not at all certain that the increases in blood pressure which have been observed are outside the normal range Furthermore, it has not been demonstrated that a significant rise of the blood pressure can hasten the development of experimental cholesterol arteriosclerosis or increase its severity

There are recorded in the literature a few experiments purporting to show that increased blood pressure can accelerate the development of experimental cholesterol arteriosclerosis Starokadomsky and Ssobolew¹⁴⁸ and Anitschkow⁵ used the suspension method of Klotz⁷ (suspension of the animal by the hindlegs for from 3 to 15 minutes daily) in conjunction with cholesterol feeding They found fatty deposits in the arteries of their rabbits perhaps a little earlier than when cholesterol was fed without any other treatment Anitschkow⁵ also found that narrowing of the abdominal aorta by means of a ligature slightly accelerated the development of fatty lesions above the constricted point Moehlig¹ has reported that daily injections of solution of pituitary increase the severity of the vascular lesions produced in rabbits by cholesterol feeding On the other hand, Bailey¹⁹ found that neither suspension nor injections of solution of pituitary had any effect on the development of arterial lesions in cholesterol-fed guinea-pigs

It seems unlikely that any of these experimental procedures could have produced a sustained rise of blood pressure Even the extent of the assumed fluctuations of blood pressure with each repetition of the treatment is a matter for conjecture since no blood pressure determinations were made in any of the experiments Thus, it is impossible to say exactly what might have been responsible for the rather doubtful acceleration of the rate of development of the lesions in the arteries In some instances the upsets in the circulatory mechanism may have been severe enough to amount to actual trauma In any event, these experiments fall far short of demonstrating clearly that increased blood pressure can hasten the development of experimental cholesterol arteriosclerosis

(c) *Reticulo-Endothelial and Endocrine Systems*—The production of experimental cholesterol arteriosclerosis in rabbits has been found to be facilitated by splenectomy^m and by preliminary intravenous injections of india inkⁿ which were erroneously supposed to "block" the reticulo-endothelial system These results might be expected in view

(l) 103, 104

(m) 141, 142

of the fact that in cholesterol-fed rabbits lipoids accumulate in large quantities in the reticulo-endothelial cells¹⁴⁵ Consequently, a reduction of the functional capacity of the reticulo-endothelial system might well accentuate the effects of cholesterol feeding

Several investigators¹¹ have reported that castration promotes the development of experimental cholesterol arteriosclerosis in rabbits Subtotal suprenalectomy does not alter their susceptibility to the development of vascular lesions following the feeding of hydrous wool fat⁹ The contradictory results of Moehlig¹² and Bailey¹⁰ regarding the effects of injections of solution of pituitary were mentioned in the discussion of the influence of fluctuations in blood pressure

The most pronounced effects attributable to endocrine influences are those which follow interference with thyroid function Extirpation of the thyroid gland has been found to facilitate the development of experimental cholesterol arteriosclerosis in rabbits,¹¹ while the feeding of dried thyroid gland¹ or of thyroid extract⁵⁰ in conjunction with cholesterol retards the progress of the lesions Thyroxine seems to be less effective in this respect than whole thyroid gland¹⁵⁸ Thyroidectomy has little effect on the blood cholesterol curve in cholesterol-fed rabbits,⁵ but when thyroid preparations are administered with cholesterol to normal rabbits, the resulting hypercholesteremia is much less extreme than that which occurs in control animals fed cholesterol alone¹ This latter fact suggests that the administration of thyroid preparations in some manner assists the organism in disposing of exogenous cholesterol and in this way exerts its protective action against the effects of cholesterol feeding which would otherwise follow

A number of recent investigations¹¹ have demonstrated that the administration of large doses of certain organic iodine compounds or of potassium iodide retards or even prevents the development of experimental cholesterol arteriosclerosis in rabbits It has been shown further that the administration of potassium iodide greatly diminishes the hypercholesteremia which usually follows cholesterol feeding¹ The analogy to the effect of feeding thyroid gland immediately suggests the possibility that the iodine compounds act through an influence on thyroid activity The experiments of Turner and Khayat¹⁵⁹ indicate that this is actually

(n) 34, 108, 141, 142

(o) 141, 142

(p) 103, 104

(q) 141, 142

(r) 109, 158

(s) 50, 159

(t) 50, 158

(u) 88, 90, 106, 116a, 139, 158, 159

(v) 139, 158, 159

the case, for they found that the prophylactic effect of potassium iodide is abolished by thyroidectomy¹¹

(f) *Injury to the Arteries*—It was mentioned in describing the microscopic features of experimental cholesterol arteriosclerosis in rabbits (section IV b) that certain definite alterations occur at the sites of fatty deposits prior to the appearance of lipoids in those areas. The alterations in the intima are inconspicuous, but those in the media can hardly be overlooked and are of such a nature as to be identified as the result of some sort of injury. The fact that these changes precede the appearance of stainable fat is in itself suggestive of a causal relationship. It seems not unlikely that these preliminary changes are responsible for the subsequent accumulation of lipoids in the areas so altered. It is even possible that these preliminary alterations may be indispensable to the process of lipoid accumulation, and evidence in support of this possibility is forthcoming from the experiments to be described in subsequent paragraphs. However, before these experiments are considered, certain time relationships should be called to mind.

In discussing the rôle of cholesterol in the diet, it has been emphasized that a certain minimum period of time must elapse before fatty deposits will make their appearance in the arteries of animals subjected to the administration of cholesterol, even though large doses are employed. In rabbits, lipoid deposits in the arteries have never been produced, so far as I am aware, within less than 20 days from the commencement of experiments which involved no procedure other than the feeding of a cholesterol-rich diet. This lapse of time might conceivably depend on a necessity for building up a sufficient concentration of cholesterol in the blood stream, but the cholesterol content of the blood rises markedly shortly after the commencement of feeding, and before stainable fat appears in the arterial walls, it has usually reached a value of 3 or 4 times the normal, a degree of hypercholesteremia which is known to be much greater than is essential for the development of lipoid deposits in the walls of the arteries. On the other hand, it may be supposed that the delay in the appearance of lipoids in the arterial walls is due to a necessity for the prior development of the alterations in the walls of the vessels mentioned in the preceding paragraph. The latter idea gains support from the fact that experimental injury to the arteries of cholesterol-fed rabbits makes possible the accumulation

(v¹) The recent experiments of Rosenthal (Arch Path **18** 827, 1934), combining the administration of cholesterol and that of relatively small doses of iodides in rabbits, yielded results which are quite the opposite of those obtained with massive doses of iodine compounds as outlined. Histologic examinations of the thyroid glands in his animals indicated that the much smaller doses of iodides had decreased thyroid activity. This effect on the thyroid, presumably the reverse of that produced with large doses of iodides, was held responsible for the apparent discrepancy in the experimental results.

of stainable fat in the injured areas in much less than 20 days, or, if the experiment is of longer duration, it permits a much more abundant deposit of lipoids in the damaged regions than in the remaining parts of the arterial tree

Ssolowjew¹⁴⁵ carried out a series of cholesterol feeding experiments on rabbits in which the carotid artery and aorta were damaged in small areas by cauterization of the adventitial surface. When cauterization was performed at the commencement of the feeding or after it had been in progress for some time, abundant lipoid material was found in the injured parts of the media as a diffuse deposit of fine fatty droplets. This was the case even within 9 days after cauterization, when cholesterol feeding had been carried on for some time previously. The same observation was made at the end of 14 days after a cauterization which was performed coincidentally with the commencement of the cholesterol feeding. In this case no trace of a lipoid deposit was found elsewhere in the arteries.

In a later series of experiments,¹⁴⁶ Ssolowjew found that similar results followed injuries produced in a different way. Rabbits were prepared by an operation in which the carotid artery was dissected free and brought out to a position just beneath the skin. It was found that this procedure with or without subsequent massage of the artery produced tiny transverse tears in the inner part of the wall of the vessel. When animals prepared in this way were fed with cholesterol in oil, the injured areas in the carotid artery, if not completely healed, became impregnated with lipoids, while the adjacent normal parts of the vessel remained free from stainable fat. The fatty material was deposited in relation to the intercellular ground substance in the damaged parts of the media, and fat was also contained in scattered phagocytic cells. In one other experiment in which cholesterol feeding had been started some time before operation, the manipulated carotid artery showed lipoid accumulations in the injured areas at the end of only 4 days from the time of operation, although the opposite carotid artery which had been left undisturbed showed no lipoid deposits.

Perhaps at this point a less artificial type of local injury may be brought into consideration for its localizing and accelerating effect on the accumulation of lipoids in the walls of arteries. Such injured areas are conveniently furnished by the occurrence of the common medial variety of spontaneous lesion in the aortas of rabbits. As has been mentioned, these areas of necrosis and calcification in the media are always characterized by an almost complete absence of stainable fat. However, when cholesterol feeding is instituted, the injured areas, excepting their calcified parts, rapidly become impregnated with an abundant deposit of fine fatty droplets or granules. Thus, in one of my own experiments,⁴⁵ a spontaneous medial lesion in the aorta of a rabbit showed a

diffuse deposit of fine anisotropic lipid granules at the end of 14 days of cholesterol feeding. Another animal showed a most abundant deposit of anisotropic fatty material in a similar medial lesion after only 25 days of feeding. In neither of these animals was there any trace of lipid deposit in the remaining parts of the arteries. With a diet similar to that used in these rabbits, the earliest fatty deposit appeared in an otherwise normal aorta only after 38 days. After protracted feeding of cholesterol in rabbits, an area of spontaneous medial necrosis is marked out even in the gross by a fat-laden circular thickening of the intima, which microscopically shows the features characteristic of the intimal lesions of experimental cholesterol arteriosclerosis. The injured media beneath shows dense lipid infiltration.

All of these experiments demonstrate clearly that the damaged tissue, especially the altered intercellular ground substance, in injured areas in the walls of arteries has a marked predilection for the accumulation of lipoids. The injured tissues become infiltrated with lipoids in a much shorter time than is necessary for the development of a similar infiltration in a previously undamaged vessel. Furthermore, it is clear that hypercholesteremia (together with any other necessary changes in the blood) of sufficient degree to permit the deposition of lipoids in the arterial walls can exist without the development of any lipid deposits in normal parts of the arteries. Under such conditions at least, injury to the wall of the vessel proves to be an indispensable factor without which lipid deposits cannot or do not occur.

The arteries of rabbits can be injured also by injections of a variety of substances, and this means may be employed for the purpose of studying the effects of injury on the development of experimental cholesterol arteriosclerosis. Intravenous injections of epinephrine and nicotine have been used in this way.^(w) Either epinephrine or nicotine alone can produce severe lesions in the rabbit's aorta simulating very closely those which follow the administration of barium chloride, hydrastine, digitalin, diphtheria toxin, etc.^x The exact way in which the injury is produced is still uncertain, but it seems probable that these substances act more or less directly on the arteries rather than through an effect on the blood pressure.^y The most conspicuous features of the lesions in the aorta are necrosis and subsequent calcification of the media. Changes in the overlying intima are relatively slight but definite, consisting of a swelling of the subendothelial ground substance and sometimes fraying of the internal elastic lamina. Intimal proliferation may occur in more advanced lesions, but throughout the process there is an almost complete absence of stainable fat in both the intima and the media.

(w) 5, 37

(x) 71, 127

(y) 37, 71, 83, 127

When injury of this sort is combined with cholesterol feeding in rabbits, the accumulation of anisotropic lipoids in the walls of the aorta is considerably accelerated, as has been shown by Amitschkow⁷ and Danisch³⁷. In their experiments, in which epinephrine and nicotine were the damaging agents employed lipid deposits were constantly associated with the lesions caused by the epinephrine or the nicotine. The lipid deposits were found in the necrotic parts of the media and in the swollen subendothelial layer of the intima over the medial lesions.

The administration of large doses of irradiated ergosterol (viosterol, vigantol) also is capable of injuring the aortas of rabbits, producing lesions almost identical with those which follow repeated injections of epinephrine. This fact was first demonstrated by Kreitmair and Moll⁷⁹ in 1928, and their observation has since been confirmed by many others.

Schmidtman¹³⁴ found that the administration of vigantol in conjunction with cholesterol feeding promoted the development of experimental cholesterol arteriosclerosis in rabbits. Her observations were confirmed by Pfeiderer¹²¹ in a more extensive study. In his experiments, the most abundant deposits of lipoids in the walls of the arteries were always found in association with the easily recognizable vigantol lesions, the severity of which seemed to govern the abundance of the lipid accumulations. Pfeiderer concluded that the arterial injury produced by the vigantol had caused a greatly increased predilection for the accumulation of lipoids in the arterial walls.

Harrison's experiments⁵⁹ with the combined administration of viosterol and cholesterol were arranged somewhat differently and the results demanded a somewhat different interpretation. His rabbits were given a preliminary course of viosterol and calcium lactate by mouth. This treatment produced densely calcified localized lesions in the media of the aorta. The administration of viosterol was discontinued, and after an interval of ten days a prolonged course of cholesterol feeding was instituted. At the termination of the experiments, the old calcified viosterol lesions showed no lipid deposits except around their margins, in the parts of the aortic wall immediately adjacent, where the typical fatty lesions attributable to cholesterol feeding were constantly located. The explanation offered for this peculiar localization seems plausible. Harrison argued that the vessel wall at the margins of the rigid calcified plaques must have been subjected continually during life to mechanical distortion occasioned by the systolic distention and diastolic recoil of the aorta. He concluded that these excessive movements within the vessel wall were responsible for the localization of the cholesterol lesions in these areas. It may be thought that this explanation necessitates the recognition of a mechanical factor as a predisposing and localizing influence quite distinct from other forms of injury to the walls of arteries. However, Harrison's conclusion really implies only this, that sufficiently violent mechanical distortion of the arterial wall can produce

a local injury which is adequate to determine the localization of experimental cholesterol arteriosclerosis

There is some evidence to indicate that bacterial infections may also have an influence in promoting the development of experimental cholesterol arteriosclerosis in rabbits. Saltykow¹²⁹ carried out a large number of experiments on rabbits which were fed on milk and bread and which, in addition, were subjected to intravenous injections of old cultures of staphylococci. The cholesterol content of milk is not high, but the experiments extended over periods up to 2 years, so that the animals probably received a large quantity of cholesterol in that time.¹²⁸ Saltykow believed that the typical fatty arterial lesions which he produced were caused primarily by the cholesterol contained in the milk but he concluded that the injections of staphylococci had a definite effect in accelerating their development and increasing their severity. This conclusion is supported by the recent results of Benson, Smith and Semenov.²⁴ These investigators fed a group of rabbits with cholesterol for 9 months, the total quantity fed averaging 65 Gm. for each animal. No arterial lesions were found in this group, evidently because the cholesterol was administered as a dry powder mixed with oatmeal and was therefore probably not absorbed in sufficient quantity to produce lesions by itself. However, the same daily dose of cholesterol given in the same way to another group of rabbits from the same litters as the first produced typical fatty lesions of the intima in a large proportion of the rabbits within 7 months when the cholesterol feeding was accompanied by intravenous injections of streptococci. The animals received a total quantity of cholesterol averaging only 46 Gm. for each. Injections of streptococci alone did not produce similar lesions, but frequently did result in visible injury to the arteries in the form of an acute arteritis.

The experimental results which have been quoted in the preceding pages are in agreement in showing that the development of experimental cholesterol arteriosclerosis in rabbits is facilitated by the introduction of various noxious agents which have in common the ability to injure the arterial walls as demonstrated in control experiments.

It seems evident that the alterations in the walls of the vessels attendant on the injury were responsible for the more rapid development of the lipid deposits, since the latter were found constantly in association with whatever visible lesions were present in the arterial walls. For example, in the experiments with epinephrine and nicotine, the fatty deposits in the intima and media occurred especially in those parts of the aorta which showed the characteristic epinephrine or nicotine lesions. However, the changes in the intima in such areas up to the time of the earliest appearance of lipid deposits there, were not more marked than those which have been described as occurring in the early stages of the lesions which result from cholesterol feeding alone, namely, a swelling of the

subendothelial ground substance and sometimes fraying of the internal elastic lamina. Thus, the fact that histologic changes in the intima prior to the advent of lipid deposits are not striking and conspicuous does not preclude the possibility that they represent the result of definite injury whether a known damaging agent has been administered or not.

The results of all recorded experiments which bear on the question of the influence of injury to the arteries are in agreement. They indicate that injury, however produced, will facilitate the development of lipid deposits in the arteries of rabbits fed cholesterol-rich diets. Injury to the arteries in conjunction with cholesterol feeding is able to bring about a much earlier initiation of the process of lipid deposition than ever occurs following cholesterol feeding alone. It has been shown that the general conditions in the blood may be suitable for the development of lipid deposits without the occurrence of any such deposits in normal parts of the arteries. Lipoid deposits occur especially in relation to the altered intercellular ground substance in injured portions of the walls of the vessels, and they develop much more rapidly in such areas than in previously undamaged parts of the arteries. However in the intima of the rabbit's aorta the changes found at the time of the earliest appearance of lipid deposits are the same in kind and degree whether cholesterol has been administered with or without an additional agent which is known to be injurious to the rabbit's arteries and which accelerates the deposition of lipoids in the arterial walls.

Correlation of all of these facts can be accomplished only by assuming that lipid deposits in the arteries are always preceded by preliminary local alterations in the walls of the vessels which are indispensable to the formation of the lipid accumulations, and which therefore constitute the initial stage of the development of experimental cholesterol arteriosclerosis. Moreover, it seems highly probable that these primary local changes in the arterial walls represent the result of some sort of injury. These conclusions are strongly suggested by the evidence at hand, and they are confirmed by other considerations to be discussed in a subsequent part of this paper (section VIII). I have already pointed out the probability that hypercholesteremia of itself is not solely responsible for the development of experimental cholesterol arteriosclerosis, and that some other factor is also necessary. On the basis of the evidence adduced here, it seems highly probable that some form of injury to the arterial walls is the second essential factor which operates in conjunction with hypercholesteremia in the production of experimental cholesterol arteriosclerosis in rabbits.

VII SUMMARY OF IMPORTANT EXPERIMENTAL DATA

Lipoid accumulations in various organs and fatty lesions of the arteries can be produced in rabbits by feeding diets which have a con-

siderable content of cholesterol. The general distribution of the arterial lesions has been indicated, and the aortic lesions have been described in detail in section IV of this paper. The individual lesions are discrete and have a yellowish color due to the lipoids which accumulate in them. The lipid deposits are usually to be found first in the intima in the form of minute fatty droplets lying in the subendothelial ground substance, which has previously assumed a greatly swollen appearance. The earliest lipid accumulations sometimes occur in the inner layers of the media where focal areas of muscle necrosis appear, and subsequently become impregnated with lipoids. However, the medial lesions are usually associated with corresponding changes in the overlying intima. Much of the fatty material, whether in the intima or in the media, is anisotropic. Soon after the advent of lipoids in the intima, large fat-containing phagocytic cells ("foam cells") and fibroblasts appear in varying numbers, producing an increasing thickening of the intima. The lesions in the media may become extensive through progressive destruction of muscle and elastic tissue followed by lipid impregnation of the necrotic areas. If cholesterol is withdrawn from the diet, well developed lesions in the intima take on a more fibrous character, and the lipoids gradually diminish in abundance. These lesions of "experimental cholesterol arteriosclerosis" are not dependent on spontaneous disease of the arteries for their development.

The feeding of cholesterol-rich diets probably can produce similar lesions in the arteries of guinea-pigs, and the development of these lesions, too, is thought to be independent of spontaneous arterial disease. The lesions found in the arteries of pigeons, chickens, parrots, mice, rats and goats following cholesterol feeding do not occur with any constancy, and they cannot be freed from a strong suspicion of spontaneity. They are therefore excluded from further consideration. All attempts to produce lesions in the arteries of cats, dogs, foxes and monkeys by feeding cholesterol-rich diets have been entirely without success.

In rabbits, experimental cholesterol arteriosclerosis can be produced by feeding solid cholesterol, it has never been produced with the feeding of any other lipid or with sterol-free diets. It is concluded, therefore, that cholesterol feeding is an essential factor in the production of the arterial lesions. However, the concomitant feeding of neutral fats and other lipoids greatly accelerates the development of experimental cholesterol arteriosclerosis and hence is an important factor. The factor of time is also of importance since even microscopic fatty deposits in the arteries have never been produced in less than 20 days by feeding cholesterol-rich diets and usually a considerably longer time is required.

During the course of most experiments with cholesterol feeding in which lesions in the arteries are produced marked hypercholesteremia

occurs, but the severity of the arterial lesions does not run strictly parallel with the elevation of the level of the blood cholesterol. Indeed, well marked hypercholesteremia can develop following cholesterol feeding without producing any lesions in the arteries. On the other hand, experimental cholesterol arteriosclerosis may appear in the presence of a relatively slight increase in the cholesterol content of the blood. However, it has not been demonstrated that the characteristic arterial lesions can be produced without any elevation of the blood cholesterol above its normal level. It is assumed, therefore, that some degree of hypercholesteremia is an essential factor in the development of experimental cholesterol arteriosclerosis in rabbits. The hypercholesteremia which follows cholesterol feeding is accompanied by a corresponding elevation of other lipoids in the blood, a rough index of which is given by the increase in its cholesterol content. The total result is hyperlipemia, i. e., an increase of all the blood lipoids above their normal limits.

At present there is no satisfactory evidence to substantiate the idea that the feeding of protein in conjunction with cholesterol facilitates the development of experimental cholesterol arteriosclerosis.

Experimental cholesterol arteriosclerosis can develop in rabbits without any rise of the blood pressure beyond the normal limit. Furthermore, there is no good evidence to show that an increase of the blood pressure can affect the progress of the lesions in the arteries.

Splenectomy, castration and thyroidectomy are said to accelerate the development of experimental cholesterol arteriosclerosis in rabbits. The administration of dried thyroid gland or of other thyroid preparations retards the progress of the arterial lesions. The administration of potassium iodide and of certain organic iodine compounds has a similar effect, but this is abolished by thyroidectomy.

If the feeding of cholesterol-rich diets is combined with some other procedure which is known to injure the arteries, the development of experimental cholesterol arteriosclerosis is greatly accelerated. This is true of all forms of injury which have been employed. Deposits of anisotropic lipoids occur especially in relation to the altered intercellular ground substance in the injured portions of the wall of the vessel, and they develop much more rapidly in these areas than in previously undamaged parts of the artery. Such injured areas can become impregnated with lipoids during a period in which no lipid deposition has occurred in the remaining normal parts of the wall of the vessel. It seems highly probable that some form of injury to the arterial walls is the second essential factor which operates in conjunction with hypercholesteremia in the production of experimental cholesterol arteriosclerosis in rabbits.

[The bibliography will be found at end of the last instalment, in the August issue.]

(To be concluded)

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc—Marcos Fernan-Nunez, professor of pathology and bacteriology in Marquette University, Milwaukee, has been elected a member of the National Academy of Medicine of Spain

According to *Science*, Simon Flexner, director of the laboratories of the Rockefeller Institute for Medical Research since the opening of the institute in 1903, has presented his resignation, to take effect on the appointment of his successor

On the retirement of Milton J. Rosenau from active teaching as professor of preventive medicine and hygiene in the Harvard University Medical School, his portrait by Jacob Binder has been presented to the school by his colleagues

The Lister Medal for 1936, which is awarded in recognition of distinguished contribution to surgical science, has been granted to Sir Robert Muir, professor of pathology in the University of Glasgow

Perry J. Melnick, formerly chief resident pathologist in the Cook County Hospital, Chicago, has accepted the position of pathologist in the Decatur and Macon County Hospital, Decatur, Ill

Howard W. Florey, professor of pathology of the University of Sheffield, has been appointed professor of pathology at the University of Oxford

At the London Hospital William Bulloch has resigned from the Goldsmiths' Company's chair of bacteriology and has been succeeded by S. P. Bedson

Nine fellowships in medicine, including two renewals, for study in the United States and abroad during the year 1935-1936 were awarded at the spring meeting of the Medical Fellowship Board of the National Research Council in Washington, D. C. The successful candidates are Oscar E. Block, Jr., Berry Campbell, Jack M. Curtis (renewal), Windsor C. Cutting, Samuel Gurin, Robert E. Johnson, Benjamin F. Miller (renewal), E. Byron Riegel, Morris F. Shaffer

Society News—The fifth annual convention of the Biological Photographic Association will be held at the Stevens Hotel, Chicago, on September 12, 13 and 14. Further details and copies of the program may be had by writing Ralph P. Greer, chairman of the program committee, Box 266, Hines, Ill

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

THE CORRELATION OF MINERAL METABOLISM AND THE VEGETATIVE NERVOUS SYSTEM IN THYROID DISEASE JACOB KLEIN, *Ann Int Med* 8 798, 1935

On the assumption that in thyrotoxic conditions there is a negative calcium balance that may explain the alterations in tone of the vegetative nervous system Klein studied the thyroids of fifty rats under varying dosages of iodine, calcium and epinephrine. Noting the parallelism between the iodine content and the size of the follicles, he took the average measurement of 100 follicles as the "follicular index." Normal rats had a follicular index of 251 microns, those with a diet deficient in iodine, 48 microns, those given a diet deficient in iodine plus a daily stimulation with epinephrine, 187 microns, those receiving 3 per cent calcium in addition to the iodine-deficient diet and epinephrine, 211 microns. It is concluded (1) that a dietary deficiency of iodine causes atrophy of the thyroid follicles and loss of colloid, (2) that stimulation of the sympathetic nerves with epinephrine in the presence of a deficiency of iodine causes hyperactivity of the thyroid epithelium, and (3) that administration of calcium promotes storage of colloid and neutralizes the harmful effects of the stimulation of the sympathetic nerves.

FRANK R. MENNE

THE EFFECT OF SODIUM SALTS IN SUSTAINING THE SUPRARENALECTOMIZED DOG G. A. HARROP ET AL., *J Exper Med* 61 839, 1935

A group of experiments is reported in which bilaterally adrenalectomized adult male dogs were maintained in apparently normal condition over prolonged periods, up to five months, without use of any adrenal gland preparation or extract by administration of sodium chloride and sodium bicarbonate alone. Withdrawal of the salts produced typical adrenal insufficiency. Further evidence is presented in these experiments in support of the view that the adrenal cortical hormone in the adult male dog is concerned with the regulation of sodium excretion by the kidney and thus eventually with the proper maintenance of water balance in the organism. It has no direct influence on carbohydrate metabolism. The reciprocal changes in the plasma concentrations of urea and potassium which take place as the concentrations of plasma sodium and chlorides vary are pointed out as furnishing a mechanism whereby abrupt alterations in osmotic pressure are dampened and the volumes of fluids in extracellular and intracellular compartments more efficiently stabilized.

FROM THE AUTHORS' SUMMARY

HISTOLOGIC CHANGES IN THE KIDNEYS OF ADRENALCTOMIZED RATS S. L. SIMPSON and V. KORENCHESKY, *J Path & Bact* 40 483, 1935

In adrenalectomized rats degeneration of the second convoluted tubules with some degree of consequent regeneration was found. Daily subcutaneous administration of cortical extract diminished or prevented these changes. Of two recovering rats one presented normal kidneys and the other moderately severe changes, suggesting that apparent recovery may be associated with latent adrenal insufficiency.

FROM THE AUTHORS' SUMMARY

THE THYMUS IN CHEMICAL INTOXICATIONS G. WORMS and H. P. KLOTZ, *Ann d'anat path* 11 1 1934

The changes in the thymus gland following injections of phosphorus, mercuric bichloride, methyl alcohol and arsenic were studied in dogs, rabbits and guinea-

pigs The most extensive studies were made with phosphorus, but in general the action of all the agents was fundamentally the same Atrophy of the thymus takes place by a series of stages If weakly toxic doses are injected over a long period, these stages can be observed in succession First there is an infiltration of the normally clear medullary zone by lymphocytes escaping from the intralobular artery At the same time the cortical substance becomes cleared because of the destruction of a large number of the epithelial thymic cells, resulting in an inversion of the lobule If the toxic agent is now withdrawn the thymus gradually returns to its original state In prolonged administration of the poison, however, the thymic lobules become segmented into smaller and smaller subdivisions by trabeculae of a colloid substance derived from the necrotic thymic cells There is no fibrosis or fatty change Hassall's corpuscles usually disappear also The changes resemble those produced by the x-rays and other agents

PERRY J MELNICK

RENAL LESIONS PRODUCED BY GOLD SALTS A KALLO, *Ann d'anat path* **11** 21, 1934

Eight different types of gold salts were administered to sixty guinea-pigs, rabbits and rats Normal as well as tuberculous animals were used The dosages included therapeutic, toxic and fatal doses The kidneys were examined microscopically The effects of toxic and fatal doses consisted, on the one hand, of intense hyperemia, diapedetic hemorrhages and cellular infiltration and, on the other hand, of marked degenerative changes and even necrosis of the tubular epithelium, especially of the convoluted tubules These findings were always associated with albuminuria or suppression of the urine Therapeutic doses produced mainly circulatory changes, but also slight parenchymatous changes The author therefore advises caution in the therapeutic use of gold salts

PERRY J MELNICK

DEFICIENCY OSTEITIS IN CHICKENS KEPT IN CAGES AND ITS RESEMBLANCE TO OSTEITIS FIBROSA WITH HYPERTROPHY OF THE PARATHYROID C OBERLING and M GUERIN, *Ann d'anat path* **11** 97, 1934

In eighty chickens kept in cages for varying periods up to a year marked deformity of the skeleton was observed, of a type familiar to chicken raisers and variously designated as rickets, osteoporosis or osteomalacia Etiologically, sunlight and vitamin D were not necessarily of importance The factor involved was an absence of gravel in the ration, apparently a mineral deficiency The bones were deformed, swollen, hyperemic and soft so that they were easily cut, and often had many cysts Microscopically, two distinct types of lesions were found One type resembled rickets and osteomalacia, with gradual atrophy of the bony trabeculae under the influence of the dilated and encroaching vascular spaces Sometimes only atrophy was seen, in other cases osteoid tissue was laid down in an attempt at regeneration Most of the chickens in which this type developed had been kept in darkness The second type of lesion resembled osteitis fibrosa The narrow bony trabeculae presented many Howship's lacunae associated with many osteoclastic giant cells and a cellular inflammatory tissue Cysts were frequent in this type Occasionally both types of lesions were found in the same fowl In most cases of both types there was an associated hypertrophy of the parathyroid glands Microscopically, these glands were either typical, atypical or adenomatous, the structure could not be correlated with the type of lesion

The authors believe that the different types should not be considered as separate entities Separate etiologic factors have not been established for them They are different reaction forms of bone tissue depending on various factors The parathyroid hypertrophy is considered as secondary to the disturbance of the mineral metabolism and reversible In osteitis fibrosa the stimulus to hypertrophy results

in an adenomatous proliferation which is irreversible. Thus, rickets, osteomalacia and osteitis fibrosa are related in that each fundamentally is a disturbance of mineral metabolism

PERRY J MELNICK

NEW FORMATION OF CAPILLARIES IN INFLAMMATORY TISSUE J GOLDNER, *Ann d'anat path* **11** 461, 1934

Inflammatory tissue produced aseptically by injecting foreign material intraperitoneally into guinea-pigs was fixed in Zenker's fluid, sectioned and stained with silver stains as well as by hematologic methods. Goldner considers three factors to be important in the new formation of capillaries in such tissue, namely, reticulin fibers, histiocytes and endothelium-formative cells. The reticulin fibers are of primary importance. They are usually laid down by histiocytes and determine the location and direction of the new-formed capillary, that is, they form a kind of framework for it. The histiocytes remain as an incomplete perithelial sheath or adventitia (cells of Marchand). The endothelium-formative cells, which may be either fibroblasts or monocytes, then form an endothelium for the capillary. Finally, secondary anastomosis of the new capillaries with the blood vessels in the periphery occurs. This evidence seems to contradict the classic theory that new capillaries bud off from preexisting ones.

PERRY J MELNICK

STATES OF INHIBITION OF THE BONE MARROW J E W BROCHER, *Ann d'anat path* **11** 585, 1934

Brocher points out that agranulocytosis is not a specific disease (so-called agranulocytic angina of Schultz), but is a state of inhibition of the bone marrow produced by various acute infections and by poisons. He reports several cases in which typical agranulocytosis (sometimes associated with hemorrhage) developed in patients following treatment with gold salts, bismuth or arsenicals or after prolonged exposure to benzene and other volatile organic poisons, x-rays and radio-active substances. The condition depends on failure of the normal reactivity of the bone marrow.

PERRY J MELNICK

EFFECT OF HYPOPHYSECTOMY ON THE PHALANGES OF THE SALAMANDER K SCHAEFER, *Virchows Arch f path Anat* **293** 562, 1934

In larval salamanders at the time of metamorphosis the toes become actually shortened. This is due to chondrolytic atrophy of the cartilaginous mass at the distal end of the terminal phalanges. Hypophysectomy of the larvae leads to abnormal enchondral ossification followed by lacunar resorption, in this process many giant cells are formed. Regeneration of amputated extremities or toes is delayed in hypophysectomized animals.

O T SCHULTZ

MORPHOLOGIC EFFECTS OF SPLENIC AUTOLYSATES G DERMAN and S LEITLS, *Virchows Arch f path Anat* **293** 599, 1934

Repeated injection of the thermostable products of autolysis of splenic tissue into rabbits and guinea-pigs led to activation of the reticulo-endothelial system of the spleen and liver. This action was increased if the splenic tissue was irradiated before autolysis. Autolysates of irradiated and nonirradiated splenic and muscle tissue caused regenerative changes in the bone marrow with increase in the number of megakaryocytes and slight degenerative changes in the parenchyma of the liver and kidney, these changes are considered nonspecific. The autolysate of the spleen of a patient with myeloid leukemia led, in the rabbit, to marked proliferation of the reticulo-endothelial system of the liver and spleen and to areas of myeloid metaplasia in the liver. The autolysate of the spleen of a patient with Banti's disease caused fibrosis of the liver in the rabbit.

O T SCHULTZ

Pathologic Anatomy

HISTOLOGY OF THE MIDOLIVARY REGION OF THE MEDULLA OBLONGATA IN THE NEW-BORN INFANT MYRTELLE M CANAVAN and FRFDERICK A HEMSATH, Am J Dis Child **49** 101, 1935

The vessels seem inadequate in number to nourish so important a structure as the medulla oblongata. In the new-born infant ependymal cells are of greater length than in older persons, averaging 20 microns. Subependymal glia cells are more abundant in the new-born than in the aged. Oligodendroglia cells are slightly more in evidence midway between the olivary bodies, where myelin is laid down at an early stage. In ten premature infants these cells represented 55 per cent of the cells, in ten babies born at term, 48 per cent. Choroid plexuses may have various foci of heaping up of epithelial cells in their tufts. Many cells are present at the surface of the folia of the cerebellum in the new-born.

AUTHORS' SUMMARY

ENLARGMENT OF THE PARATHYROIDS IN RENAL DISEASE A M PAPPINHEIMER and S L WILENS, Am J Path **11** 73, 1935

The mean weights of the parathyroid glands in a series of patients over the age of 10 years with miscellaneous non-nephritic diseases were 27 mg for the upper and from 31 to 32 mg for the lower gland. The mean combined weight was 118 mg. In the male glands there was no change correlated with advancing age. In the female gland there was an increase in weight of approximately 22 per cent during the active sexual period, after 45 years there was a decline of weight to figures corresponding with those of the series as a whole. The enlargement was not correlated with pregnancy. The mean weight of the parathyroids in various types of chronic renal disease exceeds that in non-nephritic diseases. In an unselected series of cases this increase in mean weight is approximately 50 per cent in cases of advanced renal lesions the increase amounts to more than 100 per cent. The increase in the weight of the parathyroids is roughly proportional to the severity and extent of the renal lesions and to the intensity of the clinical signs of renal insufficiency. Usually three or four of the glands share in the enlargement.

FROM THE AUTHORS' SUMMARY

ATYPICAL AMYLOID DISEASE D PERLA and H GROSS, Am J Path **11** 93, 1935

Three unusual cases of amyloid disease are reported in which an etiologic factor was not demonstrated. The first was that of a woman, 53 years of age, with extensive amyloid disease of the heart, tongue, gastro-intestinal tract and other organs, who died of congestive heart failure. The second was that of a girl, 16 years of age, with extensive amyloid deposits in the kidneys, liver and adrenals, who died of uremia. She had ankylosis of one joint without any evidence of suppuration. The third case was that of a woman, 63 years of age, with amyloid contracted kidneys, who eventually died of uremia. She had severe coronary sclerosis with old occlusion of the descending branch of the left coronary artery and healed infarction of the left ventricle. The amyloid disease was limited to the kidneys. In view of the absence of any apparent suppuration it is suggested that this peculiar disturbance in protein metabolism may be independent of destruction of tissue.

FROM THE AUTHORS' SUMMARY

HISTOGENESIS OF SUBACUTE LYMPHATIC LEUKEMIA J STASNEY and H DOWNEY, Am J Path **11** 113, 1935

With three biopsies taken at different times, the histogenesis of a case of subacute lymphatic leukemia was followed. In the early stages, a lymph node from the inguinal region showed diffuse hyperplasia of the syncytial reticulum cells. Many of these cells showed beginning basophilia in the cytoplasm. At the same

time the peripheral blood contained a number of larger cells with cytoplasm of a type that is characteristic of the reticulo-endothelial cell, but with a nucleus of lymphocytic pattern, indicating the heteroplastic origin of lymphocytes from the reticulum. Later an inguinal lymph node presented a dense mass of immature lymphocytes in the medullary region, while the cortical germ centers were still preserved. The immature lymphocytes, therefore, originate in the medulla rather than in the germinal center of the follicles, and the diffusely distributed syncytial reticulum is the mother tissue for these immature lymphocytes.

FROM THE AUTHORS' SUMMARY

HEPATO-ADRENAL NECROSIS WITH INTRANUCLEAR INCLUSION BODIES G M HASS,
Am J Path **11** 127, 1935

The case of a 7 months premature infant afflicted with a fatal disease characterized by hepato-adrenal necrosis and intranuclear inclusion bodies in the parenchymatous cells of the liver and adrenal cortex is described. It is assumed that the unique lesions must have been produced by a filtrable virus. No record of a similar case has been found in the literature.

FROM THE AUTHOR'S SUMMARY

MYOCARDIAL LESIONS IN SUBACUTE BACTERIAL ENDOCARDITIS O SAPHIR, Am J
Path **11** 143, 1935

Myocardial changes were encountered in thirty-five cases of subacute bacterial endocarditis. These changes may be summarized as cloudy swelling, fatty degeneration, petechial hemorrhages, acute myocarditis, foci of necrosis and abscesses, areas of perivascular acute and chronic (nonspecific) inflammation, minute infarcts, emboli in branches of the coronary arteries, Aschoff bodies and perivascular fibrosis. Minute infarcts were the most commonly encountered and the most characteristic lesions. Bracht-Wachter bodies are discussed and the conclusion reached that this term signifying specific lesions should be discarded because of the uncertainty of just what constitutes a Bracht-Wachter body.

FROM THE AUTHOR'S SUMMARY

ANNULAR PANCREAS J B McNAUGHT and A J COX, Am J Path **11** 179,
1935

Another case of annular pancreas is recorded, bringing the total number of reported cases to forty-four. Annular pancreas is undoubtedly a developmental anomaly of the ventral pancreatic anlage. Our case was that of a man, 70 years of age, with several congenital anomalies but with no complaints referable to them. A rapid and simple method of accurately tracing the duct system of the pancreas by means of an injection of a bismuth suspension is described.

FROM THE AUTHORS' SUMMARY

FRAGILITY AND MATURATION OF RETICULOCYTES CAMILLE MERMOD and W DOCK
Arch Int Med **55** 52, 1935

The reticulocytes, particularly the young (heavily stained) ones, are more easily destroyed in shed blood and in the circulating whole blood by saponin (and by citrate and oxalate in whole shed blood) than are the other red cells. The maturation of reticulocytes in the circulating blood is not proved, it seems not to occur on any significant scale. The occurrence of a reticulocytic crisis early in the treatment of anemia is probably an unavoidable evil associated with a change in the structure and function of the bone marrow. This precedes and delays the rise in the red cell count.

AUTHORS' SUMMARY

PARAPLEGIA OF PREGNANCY (SUBACUTE COMBINED DEGENERATION OF THE CORD)
 GEORGE B HASSIN and ABRAHAM ETTLESON, Arch Neurol & Psychiat
32 1273, 1934

In a girl, aged 19, who gave a history of gonorrhea and syphilis and who had been pregnant five months spastic paraplegia developed without marked paresthesia or definite sensory disturbance. No signs of pernicious anemia or of cord compression were present. The condition grew worse and after a spontaneous premature delivery of the child at the eighth month the patient died with signs of septicopyemia. Necropsy revealed far advanced degeneration of the lateral columns, foci of septicopyemia in the brain and the mild degeneration of the posterior columns and changes in the anterior horn cells typical of so-called axon reaction (swollen cell bodies, misplacement of the nucleus to the periphery, retraction of the dendrites and chromatolysis)

AUTHORS' ABSTRACT

THE CENTRAL NERVOUS SYSTEM IN MUSHROOM POISONING ELI MARCOVITZ and
 BERNARD J ALPERS, Arch Neurol & Psychiat **33** 53, 1935

The authors describe central nerve changes in a woman aged 26 and a man aged 27 who died from mushroom poisoning (*Amanita phalloides*) on the fifth and seventh days, respectively. The changes in the brain were punctate hemorrhages and swollen, fragmented and vacuolated ganglion cells, some with pyknotic nuclei and containing lipoids, which were present also in the neuroglia, microglia, vascular endothelium and ependymal cells. In addition, there were scattered inflammatory changes in the form of marked perivascular infiltrations. The authors classify the changes as toxic encephalopathy combined with inflammatory phenomena which, in their opinion, are not secondary but primary, in no way different histologically from an infectious nonsuppurative encephalitis. The temperature in one case rose as high as 106 F, in the other case it was not given.

GEORGE B HASSIN

CEREBRAL CHANGES IN GASTRO-INTESTINAL INFECTIONS WITH TERMINAL
 CACHEXIA LEO ALEXANDER and T T WU, Arch Neurol & Psychiat
33 72, 1935

Alexander and Wu studied the changes in the brains of twenty patients in whom cachexia developed as the result of various types of dysentery and intestinal tuberculosis (five cases). The ages of the patients varied from 6 months to 49 years. One patient was a premature baby. The durations of the diseases ranged from a few days to several months. Some patients had had recurrent attacks of diarrhea for as long as ten years. The clinical manifestations were in many cases psychotic—delirium, maniacal outbursts and Korsakoff's syndrome, convulsions were a prominent feature in a few cases, aside from severe somatic disturbances. The cerebral changes were mainly regressive—parenchymatous and circulatory. Hemorrhages and inflammatory phenomena were entirely absent. Ischemic foci in the third and fifth cortical layers were the most outstanding changes. In such foci ganglion cells were few, many were replaced by small glial cells, some cells disappeared without leaving traces, other cells were as shadows or were "pyknotic" or "pyknomorphic," but foci of complete ischemic necrosis were not present. The other changes were what the authors call "soaking," as they resembled the changes that can be produced in the ganglion cells by soaking fresh brain tissue in distilled water. Such changes show as marked argentophilia of the nuclei and crumbling of the neurofibrils. In five cases so-called pseudo-atrophy of the brain was observed especially of the frontal lobe, without fibrosis of the meninges, which in the authors' opinion was due to emaciation.

GEORGE B HASSIN

MENINGO-ENCEPHALITIS CAUSED BY CYSTICERCUS CELLULOSA? VIRA B DOLGOPOL and MARCUS NEUSTAEDTER, Arch Neurol & Psychiat **33** 132, 1935

A Mexican woman, aged 30, had been suffering for a year from headaches which were complicated within five months by visual disturbances, vomiting and convulsive seizures of the left arm and leg. Present were Argyll Robertson pupil, papilledema of the left fundus, edema of the eyelids, and pleocytosis and increase of globulin in the spinal fluid, which contained 24 per cent eosinophils (the blood showed 10 per cent of eosinophils). Jacksonian attacks developed in the right half of the body preceded by hyperesthesias of the right upper and lower extremities. Because of gradually increasing visual disturbances, an opening was made in the right temporal region and a small mass was removed from under the dura. Five weeks later necropsy revealed chains of vesicles attached to the meninges of the sylvian fissure and both sides of the pons, the majority of the cysts were empty or contained a hyaline substance but no parasites. The capsules of the cysts showed marked reactive inflammatory phenomena, as did the meninges and the cerebral parenchyma. The vessels of the brain were not only infiltrated with lymphocytes and plasma cells but showed a marked endarteritis which almost obliterated the lumen of some arteries. This occlusion was evidently the cause of the softening of the right parietal and temporal lobes. The National Institute of Health in Washington diagnosed the vesicles as *Cysticercus cellulosae*, the larval form of *Taenia solium*.

GEORGE B HASSIN

SIDEROFIBROSIS OF THE SPLEEN IN SICKLE CELL ANEMIA L W DIGGS, J A M A **104** 538, 1935

This article is based mainly on a study of the spleen in nineteen cases of sickle cell anemia. The descriptions of the spleen in thirty-three cases of the disease reported in the literature are also considered. The various stages of the siderotic fibrosis of the spleen in sickle cell anemia are described in detail, but no conclusions are drawn as to the relationship of the splenic lesion to the severity and duration of the anemia. The specific feature is the presence in the spleen, in all stages of the process, of sickle-shaped red corpuscles. The process is the result of hemorrhages into the spleen followed by replacement with connective tissue in which are deposits of iron and calcium. In these deposits may occur structures that look more or less like mycelial bodies, but there is no convincing evidence that fungi play any part in the process.

ELLIPTICAL HUMAN ERYTHROCYTES D J STEPHENS and A J TATELBAUM, J Lab & Clin Med **20** 375, 1935

The authors present hematologic studies of fifteen members of an Italian family in which eight members exhibited elliptic erythrocytes. In each of the affected ones there was an increase in the number of red blood cells per cubic millimeter with a definite increase in the mean corpuscular volume and the mean corpuscular hemoglobin. In general the number of red blood cells was inversely proportional to the mean cell volume and the mean hemoglobin content. Two dimensional measurements of cells in fixed, stained smears indicated that the decrease in size involved the round cells and the abnormally shaped cells to approximately the same extent, without increase in the variability of distribution of cell size. In the unaffected members of the family the erythrocytes were normal in shape, size, volume and hemoglobin content.

FROM THE AUTHORS' SUMMARY

RELATION OF ADENOMA OF THE UMBILICUS TO APOCRINE SWEAT GLANDS AND TO ADENOFIBROSIS OF THE MAMMARY GLAND H GEHRKE, Virchows Arch f path Anat **293** 191, 1934

Gehrke reviews the theories of the pathogenesis of umbilical adenoma, for which he prefers the name "adenofibrosis," and of the endometrioid proliferations in gen-

eral He finds little support for the view that the umbilical tumor arises from celomic epithelium or from metastatic endometrioid tissue He describes the histologic appearance of an umbilical nodule from a woman aged 89 and attaches great importance to the myxomatous character of the stroma about the glandular elements At the periphery of the tumor there occurred transitions from normal sweat glands to cysts and glands with columnar epithelium In the transition zone were sweat glands of apocrine type with myxomatous stroma He accepts as established the normal occurrence of apocrine sweat glands in the umbilicus of woman It is from these that he derives the umbilical adenoma Participation of such tumors in the menstrual cycle is not proof of their endometrial origin, since the apocrine sweat glands are influenced by sex hormones In cystic disease or adenofibrosis of the mammary gland the presence of large glands of apocrine sweat gland type is frequently observed The myxomatous character of the stroma about them is cited by Gehrke in support of his theory

O T SCHULTZ

HISTOLOGY OF BONE INVOLVEMENT IN PARONYCHIA G A SEDGLNIDSE, Virchows Arch f path Anat **293** 207, 1934

Two forms of involvement of the terminal phalanx in suppurative paronychia have been described (1) periostitis and involvement of the subadjacent bone by direct continuity from the infected soft tissues and (2) osteomyelitis by way of the blood stream Histologic examination of early lesions leads the author to conclude that such a difference does not exist The terminal phalanges have a much richer blood supply by way of the periosteum than other bones The inflammatory process attacks the bone by way of the periosteum, osteomyelitis may result as a late stage of this process but not as a primary process beginning in the medullary spaces of the bone The entire phalanx may die early in the infection, sequestration of part of the bone does not occur because the rich blood supply of the periosteum permits rapid regeneration if the inflammation has not been so diffuse as to cause complete necrosis in a short time

O T SCHULTZ

THROMBOPHILIA AND POLYCYTHEMIA H LUDEKE, Virchows Arch f path Anat **293** 218, 1934

The term "thrombophilia," according to Ludeke, should be reserved for a condition characterized by a pathologic disposition to multiple, not infrequently recurring, thrombosis, often in vessels not usually the site of this process, in persons who do not exhibit the factors usually held responsible for thrombosis Four cases are presented Platelet counts were not made In two cases histologic examination of the bone marrow revealed a great increase in megakaryocytes In the other two, bone marrow was not available for study, but the clinical picture was that of polycythemia Ludeke holds that all were cases of polycythemia He tabulates from the literature twenty-one additional cases of polycythemia with thrombosis Thrombosis in polycythemia is due to increased formation and delivery into the blood of platelets (thrombocytosis) and to slowing of the circulation The latter phenomenon is due to increased viscosity of the blood and to an increase in the cross-sectional area of the circulatory bed brought about by the increased blood volume The localization of the thromboses depends on local peculiarities of the circulation Polycythemia is a striking example of a pathologic predisposition to thrombosis

O T SCHULTZ

ENDARTERIAL CELLULAR BUDS OF THE THYROID A GUPIN, Virchows Arch f path Anat **293** 257, 1934

In 1892 Horne described in goitrous thyroids areas of cellular proliferation in the small arteries They projected into the lumens of the vessels as small cellular buds These findings were confirmed by M B Schmidt, who found the buds also in normal thyroids Concerning their origin and nature there has been doubt

Gilpin examined serial sections of fifty thyroids, normal and abnormal, from persons of various ages. He found the structures described by Horne. They were most numerous in the most highly vascularized thyroids. They are not true proliferations but artefacts resulting from a plane of section through twists and bends of the smaller arteries. The cellular nodules usually consist only of intima, but they may contain tissues of the other two coats as well. O T SCHULTZ

THYROTOXIC MYOCARDIAL CHANGES D BOYKSEN, *Virchows Arch f path Anat* **293** 342, 1934

Histologic examination of the hearts of twenty-five persons who died of thyrotoxicosis revealed myocardial changes in twenty-three. The detectable alterations were primarily regressive and began with loss of striation and degeneration of individual muscle fibers or small groups of fibers and proceeded to necrosis of myocardial fibers. The degenerative changes were associated with lymphocytic infiltration and with replacement of degenerated fibers by proliferated connective tissue. Causes of damage other than thyrotoxicosis were excluded. Similar changes were produced in rats subjected to subcutaneous administration of synthetic thyroxine. Myocardial damage was increased if the thyroxinized rats were exercised daily on a treadmill to exhaustion. Similar muscular activity in rats without thyroxine led to no myocardial changes. Boyksen holds that the myocardial damage of hyperthyroidism is due to thyrotoxic action on the muscle fibers.

O T SCHULTZ

PATHOLOGIC ANATOMY OF HUMAN PSITTACOSIS B J MANSFENS, *Nederl tijdschr v geneesk* **78** 5818, 1934

The lung may show lobular pneumonia with a gray or red, smooth, moist cut surface. The exudate consists mostly of serous fluid with little fibrin, some large mononuclear cells and few leukocytes. There may be fibrinous pleuritis. In the liver and kidney there is degeneration of the epithelium, and sometimes there is focal necrosis in the liver. There may be swelling of the reticulo-endothelial cells in the spleen and in the liver, also small hemorrhagic extravasations in different organs.

Pathologic Chemistry and Physics

THE BLOOD AND PLASMA GLUTATHIONE CONTENT IN HUMAN CANCER J W SCHOONOVER, *Am J Cancer* **23** 311 and 315, 1935

The average reduced and oxidized glutathione contents of the blood of patients with cancer is practically the same as in normal blood. The reduced glutathione content of erythrocytes is slightly higher than normal in the presence of both cancer and nonmalignant tumors, while that of blood plasma is low and fairly uniform. The ratio of oxidized to reduced glutathione is higher in the plasma and lower in the erythrocytes of patients with cancer than in normal persons.

ELIZABETH MCBROOM

PHOSPHATASE IN OSSIFYING MYOSITIS W E WILKINS, M REGEN and G K CARPENTER, *Am J Dis Child* **49** 1219, 1935

Sections of tissue from a patient with so-called myositis ossificans progressiva were studied for evidence of phosphatase activity. Taken from the region of two lesions in the preossification stage in a patient with progressive ossifying myositis, fibrous tissue had a much higher phosphatase activity than muscle. The activity in two samples of normal muscle was low. Samples of heterotopic bone and cartilage from an older lesion had a higher activity than a piece of normal rib.

FROM THE AUTHORS' SUMMARY

CALCIUM-ION CONCENTRATIONS IN THE BLOOD F C McLEAN and A B HASTINGS, *Am J M Sc* **189** 601, 1935

The total calcium of the serum or plasma is nearly all accounted for as calcium ions and calcium bound to protein. Of these two forms, the ionized calcium is of primary physiologic and clinical importance. The concentration of calcium ions in the plasma is normally maintained within a relatively narrow range by a process of physiologic regulation in which the parathyroid glands play a prominent part. This range has been found to be, as a rule, from 4.25 to 5.25 mg per hundred cubic centimeters. The concentration of calcium ions in the plasma at any time is the resultant of an equilibrium between the total calcium and the total protein in the plasma. A method for clinical estimation of calcium ion concentration by calculation from the total protein and the total calcium concentration is presented. Results obtained by this method have been found to be in substantial agreement with those obtained by direct observation. Fluctuations in the concentration of total calcium in response to fluctuations in that of total protein in the plasma are necessary for the maintenance of a normal calcium ion concentration, and consequently are not in themselves of clinical importance. An increase in the concentration of calcium ions in the plasma is presumptive evidence of hyperfunction of the parathyroid glands. A decrease in the concentration of calcium ions in the plasma may occur as the result of hypofunction of the parathyroid glands, or may be brought about by the hyperphosphatemia of uremia. In all other conditions investigated, including various disorders of calcification, the concentration of calcium ions in the serum or plasma has been found to be within normal limits.

FROM THE AUTHORS' SUMMARY

THE LIPID CONTENT OF LIVERS OF NON-IMMUNIZED AND IMMUNIZED HORSES A WADSWORTH, L W HYMAN AND R R NICHOLS, *Am J Path* **11** 419, 1935

The lipids in the liver of immunized and those of nonimmunized horses were determined by estimating the total fatty acids, phospholipids, free and esterified cholesterol and the iodine value of the total fatty acids. The values for the immunized showed much wider variation than did those for the normal animals. The ratio of phospholipid to free cholesterol in the liver from the immunized horses was lower than in that from the nonimmunized ones and corresponded, in general, to the changes in the liver.

GASTRIC ACIDITY IN PULMONARY TUBERCULOSIS I GRAY and J MELNICK, *Am Rev Tuberc* **31** 460, 1935

Of young persons between 20 and 30 years of age with pulmonary tuberculosis of from one to six or more years' duration, approximately one third presented gastric hypo-acidity and one fourth an acidity within a period of from one to four years. Approximately one half of the number showed a normal acidity at all times. Of male patients, a little more than half showed normal acidity through a six year period of the disease. Approximately one third showed a decrease or an absence of acid. Of female patients only one third had normal acidity, the other two thirds showing a decrease or an absence of acid. There was a definite tendency in the females toward a decrease in acid. The gastric contents of the majority of the young adults showed tubercle bacilli. As the acid decreased tubercle bacilli were found more frequently in the stomach contents. Gastric symptoms were somewhat more frequent in the females than in the males and usually started at an earlier period.

H J CORPER

RELATION OF PLASMA PROTEINS TO ASCITES AND EDEMA IN CIRRHOSIS OF THE LIVER W K MEYERS and C S Keefer, *Arch Int Med* **55** 349, 1935

Sixteen cases of cirrhosis of the liver were studied. A deficiency of total plasma protein was found. The decrease was most pronounced in the albumin

fraction The albumin-globulin ratio was inverted Similar but less extensive and less consistent alterations in the values of plasma protein were observed in fourteen cases of other forms of disease of the liver

In patients with cirrhosis of the liver the following conditions were observed
 1 The protein content of the ascitic fluid varied between 0.1 and 1.7 Gm per hundred cubic centimeters The albumin content of the ascitic fluid was proportionately greater than that of the blood plasma
 2 The removal of ascitic fluid gave rise to changes in the plasma protein value and temporary hydremia with corresponding diuresis
 3 Peripheral edema without circulatory failure was associated with a reduction of the plasma proteins

In cirrhosis of the liver the appearance of ascites must depend somewhat on the osmotic pressure of the blood plasma as well as on portal obstruction The hypoproteinemia may arise from defective formation of plasma proteins and from loss of protein into the ascitic fluid The defect may be due to alteration in the function of the liver

FROM THE AUTHORS' SUMMARY

THE UREA RATIO AS A MEASURE OF RENAL FUNCTION H. O. MOSLYNTHAL and M. BURGER, Arch Int Med 55 411, 1935

The urea ratio expressed as the percentage of urea nitrogen in the total non-protein nitrogen of the blood, or $\frac{100 \times \text{urea nitrogen}}{\text{nonprotein nitrogen}}$ is a satisfactory index of renal efficiency When the Folin-Wu filtrate (laked blood) is used for the determination of both the urea nitrogen and the total nonprotein nitrogen in the blood it is noted that the urea ratio varies with renal function as follows (a) With normal renal function the index is 44 or less, (b) with maximal impairment of renal function the index is 80 or higher, (c) with improvement in renal function, a drop in the ratio, and with progressive impairment of function, a rise in the ratio occurs The determination of the urea ratio at intervals measures progressive changes in renal efficiency and affords a reliable index of renal function regardless of the fluctuations of the total nonprotein nitrogen of the blood Although the urea nitrogen or the total nonprotein nitrogen in the blood may be at a normal level, the urea ratio reveals renal insufficiency when it exists This test may be carried out on one specimen of blood, it does not require prolonged observation of the patient or collection of urine It furnishes a numerical index of impairment of renal function and is equally applicable to patients in private practice and to those in hospitals

FROM THE AUTHORS' SUMMARY

ALKALI RESERVE IN BLOOD AND IN CEREBROSPINAL FLUID IN EXPERIMENTAL ACIDOSIS E. DE THURZO and S. KATZENFLOGEN, Arch Neurol & Psychiat 33 786, 1935

The authors studied the blood (from the heart) and the spinal fluid (obtained by cisternal puncture) of cats for carbon dioxide-combining power before and after the blood had been rendered acidotic by intraperitoneal injections of ammonium chloride (from 0.25 to 1 cc of 10 per cent solution) In some cats in order to increase the permeability of the barrier between the blood and the spinal fluid, aseptic meningitis was produced before the intraperitoneal injection of ammonium chloride The carbon dioxide-combining power was studied at various intervals after injection of ammonium chloride In eight of twenty-five experiments, acidosis of the blood was generally accompanied by a mild reduction in the carbon dioxide-combining power of the cerebrospinal fluid This was much more marked in animals in which aseptic meningitis had been additionally produced, so that the acid-base balance in the cerebrospinal fluid is, in the authors' opinion, apt to be affected by a combination of acidosis of the blood and an inflammatory process within the cerebrospinal cavity

GEORGE B. HASSIN

URINARY SILICA IN PERSONS EXPOSED TO SILICA DUST J J BLOOMFIELD,
R R SAYERS and F H GOLDMAN, Pub Health Rep 50 421, 1935

One hundred and twenty-three anthracite coal workers, twenty of whom had been out of the industry an average of seven years, were examined for urinary silica by the method of King and Dolan. The amounts of silica found in the urine varied from 0.6 to 117 mg and averaged 2.5 mg per hundred cubic centimeters. Normal persons were found to be excreting only an average of 1 mg per hundred cubic centimeters. A close correlation was found between the exposure of these men to silica dust for a specified number of years and the amount of urinary silica. A study of former anthracite coal workers showed that even after a lapse of several years away from any exposure to silica dust an increased amount of silica is being excreted by them. These findings furnish additional evidence of the etiology of the disease.

FROM THE AUTHORS' SUMMARY

THE LIPIDS IN THE LEUKOCYTES IN FEVER AND INFECTION ELDON M BOYD,
Surg, Gynec & Obst 60 205, 1935

The leukocytes undergo marked metabolic variations in pregnancy, lactation, postoperative convalescence and fever, as indicated by changes in their lipid content. There are twenty-six complete differential analyses of lipids in the leukocytes under fasting conditions in twelve cases of fever. In each analysis the lipids determined were free cholesterol, ester cholesterol, total cholesterol, phospholipid, neutral fat, total fatty acids and total lipid. Oxidative micromethods were used. The results are grouped according to the outcome of the infections. In patients who recovered from fever due to a variety of causes the white blood cells contained large amounts of phospholipid. There was generally also an increase in free cholesterol with low figures for ester cholesterol and neutral fat. In these, convalescence was accompanied by a further rise in phospholipid. Patients dying from infection had low phospholipid values in the blood leukocytes during the febrile period. It appears that the activity of the leukocytes may be measured by their lipid content, especially by the phospholipid fraction.

WARREN C HUNTER

HAEMOCHROMATOSIS H RAMAGE and J H SHELDON, Quart J Med 4 121, 1935

In hemochromatosis there is an increase of iron in all the tissues of the body with the exception of the blood, brain and colon, concerning the latter two organs, however, the statement is subject to individual exceptions. In certain organs the amount may be enormous, especially in the liver, pancreas, lymph glands, thyroid, salivary glands, pituitary, choroid plexuses and heart. The increase over normal appears to be greatest in the pancreas. The somatic muscles share in these deposits of iron. The total amount of iron deposited in the body by the time of death appears to vary from about 25 Gm to from 45 to 50 Gm. There is a slight increase of the total sulphur in certain tissues, especially in the alimentary canal. This is probably related to the deposits of hemofuscin. Spectrographic examination confirmed the results of previous chemical analyses in showing that there is an increase of copper in the liver. This increase applies to all the tissues with the exception of the kidney, small intestine and omentum. The general order of increase is between two and three times the normal. Certain of the tissues have an increase of calcium, which is best seen in the liver, thyroid, striated muscles and pancreas. Most of the tissues show disturbances in the behavior of both sodium and potassium, these metals usually swinging in opposite directions. The manganese content of the liver is about one-fourth the normal. No unusual elements were met with.

FROM THE AUTHORS' SUMMARY

Microbiology and Parasitology

AN INTERPRETATION OF THE FILTERABLE VIRUS R R HAYE, *Am J Hyg* **21** 472, 1935

"A filterable virus, as we conceive it today, is an agent of particulate nature. It is of ultramicroscopic dimensions. It passes filters that retain the vegetative forms of parasitic life. It can be passed in series on sensitive hosts. It produces characteristic inclusion bodies. It has not been cultivated on lifeless media. It would be unknown to us were it not for the changes produced in the bodies of higher animals and plants."

RELATIONSHIP BETWEEN RHEUMATIC AND SUBACUTE BACTERIAL ENDOCARDITIS
W C VON GLAHN and A M PAPPENHILMER, *Arch Int Med* **55** 173, 1935

The infection of the cardiac valves with nonhemolytic streptococci in rheumatic patients is due to implantation of the bacteria on unhealed rheumatic vegetations. The continued activity of the rheumatic disease in patients with subacute bacterial endocarditis is attested by the invariable occurrence on the valves of rheumatic verrucae which do not contain bacteria, and by Aschoff bodies in the myocardium in the same percentage of cases as in uncomplicated rheumatic carditis. Staphylococcus aureus may also be implanted on unhealed rheumatic vegetations.

FROM THE AUTHORS' CONCLUSIONS

THE FIBRINOLYTIC ACTIVITY OF HEMOLYTIC STREPTOCOCCI W S THLETT, *J Bact* **29** 111, 1935

There is a relationship between the infectivity of the beta hemolytic streptococcus for man and the fibrinolytic property of the cultures. The determination of the presence or absence of fibrinolysin in the cultures is helpful in separating strains pathogenic for man from others which probably do not cause disease in human beings.

FROM THE AUTHOR'S CONCLUSIONS

RABBIT POX H S N GREENE, *J Exper Med* **60** 427 and 441, 1934

The clinical manifestations and course of a highly contagious and acutely fatal disease designated as rabbit pox have been described. The general symptomatology varied within wide limits, and accurate diagnosis rested on the occurrence of certain characteristic lesions. The most distinctive of these lesions was a pocklike eruption which was often outspoken and widespread over the body, but sometimes was poorly defined and detectable only after careful physical examination. Other lesions depended on involvement of particular organs and gave rise to special symptoms. As a rule, in the epidemic infection the period of incubation varied between five and seven days. No evidence of transmission of the infection by animals which had recovered could be obtained. Furthermore, tests demonstrated that the reproductive capacities of most of the animals were not permanently impaired.

The most distinctive gross lesion in all organs and tissues was the small nodule or papule, which was found to consist of mononuclear infiltration and necrosis. Diffuse lesions were also found in which the infiltration was widespread and accompanied by edema, hemorrhage and extensive necrosis of the affected tissues and organs. The possibility that the diffuse lesions were due to the action of secondary invaders was considered, but the available evidence indicated that the different types, including pneumonia, represented reactions to a single causative agent. Moreover, an intimate relationship was observed to exist between lesions and small blood vessels, in which primary endothelial damage was usually apparent. The degree of vascular damage generally corresponded to the extent of the

lesion, and it is probable that this in turn corresponded to the dose of the causative agent. The close analogy between the clinical manifestations and pathologic processes of this disease and smallpox in man led to the conclusion that the disease in the rabbit is essentially the same as smallpox, and that it is probably produced by a virus closely related to that of smallpox. The available evidence indicated that the infection originated in the institute (Rockefeller Institute for Medical Research) and that it spread in atypical form or masked by some other disease until it reached the breeding colony as a clearly defined epidemic infection.

FROM THE AUTHOR'S SUMMARIES

TRANSMISSION OF KALA-AZAR BY ORONASAL SECRETIONS C. E. FORKNER and L. S. ZIA, *J. Exper. Med.* **61** 183, 1935

Patients with kala-azar, whether the symptoms of the disease are of short or long duration, almost without exception have present in their oral and nasal discharges viable, pathogenic *Leishmania Donovanii*. Evidence is presented which strongly supports the theory that kala-azar is transmitted by means of direct or indirect contact. Unequivocal proof of the important natural mode or modes of transmission of kala-azar has not yet been presented. Much more work must be done before a final solution of the problem can be accepted.

FROM THE AUTHORS' CONCLUSIONS

DIAGNOSIS OF PSITTACOSIS IN MAN BY MEANS OF INJECTIONS OF SPUTUM INTO WHITE MICE T. M. RIVERS and G. P. BERRY, *J. Exper. Med.* **61** 205, 1935

By means of intraperitoneal injections of unfiltered sputum or of filtrates of sputum into mice psittacosis in human beings can be successfully diagnosed in a large percentage of the cases and in a relatively safe manner.

FROM THE AUTHORS' SUMMARY

REPEATED ATTACKS OF EXPERIMENTAL PNEUMOCOCCUS LOBAR PNEUMONIA IN DOGS L. T. COGGESHALL and O. H. ROBERTSON, *J. Exper. Med.* **61** 213, 1935

A study has been made of repeated attacks of lobar pneumonia produced in dogs by intrabronchial injection of *Pneumococcus*, type I. Twenty-five dogs were given seventy-eight infections at intervals of from three days to nineteen months. The number of attacks to which a single animal was subjected varied from two to eleven. It was found that recovery from this experimental disease conferred on the animals increased resistance against subsequent infections, as shown by the fact that such animals regularly survived doses of culture which in dogs infected for the first time produced a fatal outcome. The recurrent attacks of pneumonia were uniformly mild, the febrile course was brief, the pulmonary lesion was usually confined to a single lobe, and bacteremia seldom occurred. There was no detectable difference between the second and the subsequent infections, which could be produced whenever desired, nor did the intervals of time between attacks appear to bear any relation to the severity of the experimental disease. Tests for acquired antipneumococcal immune substances in the blood after recovery showed that these substances were present in some animals and not in others, yet dogs without demonstrable humoral immunity appeared to be just as resistant to reinfection as those possessing it. A comparison of the pathogenesis of these secondarily induced lesions with that of the initial infection revealed certain striking differences. Secondary lesions produced in a lobe previously affected tended to evolve much more rapidly than did primary ones. They were characterized by early appearance of a generalized macrophage reaction and marked diminution in the numbers of pneumococci in the tissues or their complete absence. These changes occurred more slowly in secondary lesions initiated in hitherto uninvolved lobes. The macrophage reaction, which consists of a swelling of the fixed tissue cells (histiocytes) and a

subsequent liberation of macrophages into the alveolar exudate, is regarded as significant evidence of increased antipneumococcic resistance, since it has been observed to occur regularly at the time of recovery from the first infection and is accompanied by local disappearance of the invading micro-organisms

FROM THE AUTHORS' SUMMARY

CORYZA OF THE DOMESTIC FOWL J B NILSON, J Exper Med **61** 351 and 361, 1935

The mucous surfaces of the nasal passages and orbital sinuses appear to afford particularly favorable conditions for the development of the bacillus of fowl coryza. Injected into the nasal tract in any appreciable number, the bacilli regularly develop and may continue to exist for a considerable period in spite of an active inflammatory reaction on the part of the host. The bacillus multiplies either sparsely or not at all when injected extranasally, regardless of the nature of the cellular surface with which it is brought in contact. If the locus of injection is in communication with the upper air passages, as in the instance of the trachea, internal ear and orbital cavity, the bacilli may be carried there even in the absence of a local development and produce coryza. Introduction of the bacilli in loci not in communication with the upper air passages is followed by nasal carriage only in the case of the peritoneal cavity. Following intraperitoneal injection of the bacilli, seven of twelve birds showed these organisms in the nasal passages and, except in one instance, without accompanying inflammation.

Intratracheal, intracloacal and subcutaneous injection of living cultures of the bacillus of fowl coryza had no demonstrable effect on the susceptibility of fowl to coryza. Intraperitoneal injection was irregularly followed by definitely altered susceptibility. Growth of the specific bacillus was inhibited in the nasal tracts of approximately 70 per cent of twenty-five birds which had received an earlier intraperitoneal injection.

FROM THE AUTHORS' SUMMARIES

THE COLONY MORPHOLOGY OF TUBERCLE BACILLI K C SMITHBURN, J Exper Med **61** 395, 1935

Smooth, round, shiny, nongranular and nonspreading colonies have been observed in cultures of virulent tubercle bacilli freshly isolated from eight human sources other than sputum. The classification of six of these strains as of human type was established by inoculation of the cultures into rabbits and guinea-pigs. The 3 per cent sodium hydroxide or 6 per cent sulphuric acid frequently used in the isolation of tubercle bacilli are definitely unfavorable to the development of smooth colonies. It was observed that smooth colonies are produced in greater number when the hydrogen ion concentration of the medium (Corper's egg yolk-glycerin medium) is adjusted to a point near to but slightly on the acid side of neutral.

FROM THE AUTHOR'S SUMMARY

EXPERIMENTAL STUDIES ON ENCEPHALITIS L T WEBSTER and G L FITE, J Exper Med **61** 411, 1935

The infectious agent in fatal cases of St. Louis and Kansas City encephalitis passes Seitz pads in high dilution without appreciable loss of infectivity and traverses collodion membranes with an average pore size of 66 microns or greater. It is highly infectious for mice by intracerebral and intranasal routes but practically innocuous by subcutaneous and intraperitoneal routes. Certain strains of mice are more susceptible than others. Administered to mice intranasally, the agent causes tremors and convulsions after an incubation period of from six to seven days, followed by prostration and death in from eight to ten days. Lesions are demonstrable in the olfactory bulbs three days after infection in the pyriform lobe after four days, and in Ammon's horn after five days. In monkeys (*Macacus rhesus*) the agent provokes a mild nonfatal reaction and the development of specific neutral-

izing bodies. On passage in monkeys the virus becomes progressively weaker. In rabbits, guinea-pigs, rats and sheep it is apparently without effect. All available strains proved alike in their effects on animals and in immunologic respects. The available data enable one to conclude that the agent is a filtrable virus differing from those studied heretofore.

FROM THE AUTHORS' CONCLUSIONS

IS A SPECIAL VARIETY OF STAPHYLOCOCCUS CONCERNED IN FOOD POISONING?
J. STRITAR and E. O. JORDAN, *J. Infect. Dis.* **56** 1, 1935

As other investigators have found, there are no good criteria for the differentiation of various types of staphylococci. Biochemical, hemolytic and agglutinative characters are not closely correlated, and except for differences in the vigor of the strains it is hardly possible to maintain a sharp distinction between the albus and aureus varieties or between strains of "saprophytic" and those of pyogenic origin. The "food poisoning" strains agree with the other members of the group in not constituting a clearly marked division. There is no evidence of homogeneity in biochemical, hemolytic or agglutinative characters. The power to provoke food poisoning is not limited to any recognizable variety of staphylococci. As bearing on the possible instability of these organisms it is worth noting that strains with different biochemical and serologic characters have been isolated from the same article of food (e. g., cake) in one and the same outbreak (cf. strains 1, 3, 4, table 1). On the other hand, the unity of the strains found in some outbreaks (e. g., 142-5) is noteworthy.

FROM THE AUTHORS' SUMMARY

EXPERIMENTAL RABIES IN WHITE MICE AND ATTEMPTED CHEMOTHERAPY. A
HOYT, R. T. FISK and C. H. THIENES, *J. Infect. Dis.* **56** 21, 1935

Groups of white mice, each comprising approximately twenty-four animals, were given intracerebral injections of fixed rabies virus. They were subsequently treated with various drugs in an attempt to prevent or modify the course of the disease. The drugs employed were plasmochin, merthiolate, metaphen, bismuth violet, iodobismutol, bismarsen, tryparsamide, silver arsphenamine, Bayer 205, ethylhydrocupreine hydrochloride (optochin), pyridium, sodium arsenite (atoxyl), neostam and sparteine sulphate. The virus and the drugs were injected in amounts directly proportional to the body weight of each mouse. The experiments performed were divided into sixteen series and an adequate control group of mice was instituted in each series. The mean period of incubation and the mean length of life (after injection of fixed virus) were calculated for each group of mice. Neither of these periods differed significantly as between the treated and control groups of mice included in any one series of experiments.

FROM THE AUTHORS' SUMMARY

THE PASSAGE OF BACTERIA FROM THE LUNGS INTO THE BLOOD STREAM. W. M.
TUTTLE and P. R. CANNON, *J. Infect. Dis.* **56** 31, 1935

Suspensions of cultures of *Staphylococcus aureus*, *Bacillus prodigiosus* and *Streptococcus haemolyticus* were introduced into the lower lobe of the left lung in fourteen healthy dogs, and samples of blood from the femoral artery and lymph from the thoracic duct were cultured at frequent intervals during the first sixty minutes. In the animals given injections of cultures of *Staph. aureus* and *B. prodigiosus* both the blood and lymph remained sterile throughout the period of observation. The bacteria were demonstrable in the lungs at the end of the experiment, many engulfed by septal cells. In the dogs given injections of cultures of the hemolytic streptococcus, however, the micro-organisms quickly entered the blood stream whereas the thoracic lymph remained sterile in most instances, proving that the passage of bacteria from the lungs into the blood stream was direct rather than indirect by way of the lymphatics. It is probable that the differences in the response of the pulmonary tissues to the micro-organisms employed depended to

a large extent on the varying degrees of injury to the cellular and tissue barriers within the lungs and that the experimental bacteremia occurred because of increased permeability of these membranes induced by the virulence and toxigenicity of the bacteria

FROM THE AUTHORS' SUMMARY

THE SPECIFICITY OF THE CONJUNCTIVAL INJECTION IN MONKEYS FOLLOWING INOCULATION WITH TRACHOMATOUS TISSUE R W HARRISON, J Infect Dis 56 49, 1935

It has not been possible to induce folliculosis in monkeys nonspecifically by mechanical or chemical irritation. Follicles have not appeared after inoculation with a variety of bacteria isolated from patients with trachoma and other ocular diseases. Repeated inoculations with bacteria and protein solutions continued long enough to establish hypersensitive responses have failed to induce follicle formation. Inoculation with tissue from patients with acute conjunctivitis, spontaneous folliculosis and inactive trachoma failed to elicit a follicular response in monkeys. In animals recovered from infection by inoculation with trachomatous tissue, follicular hypertrophy was induced only by reinoculation with trachomatous material. Monkeys which did not react to nonspecific stimuli subsequently acquired typical follicles when inoculated with active trachomatous material. These findings indicate that the experimental disease in monkeys is specific for trachoma.

FROM THE AUTHOR'S SUMMARY

COCCUS FORMS OF CORYNEBACTERIUM DIPHTHERIAE T C GRUBB J Infect Dis 56 64, 1935

An irregular alternation between the appearance of bacillary and that of coccus forms of the diphtheria bacillus was found when it was aged or repeatedly transferred in various mediums. A small colony variant, or "G" colony, was observed which did not appear to be related to a life cycle or to the production of coccus forms. Coccus forms of certain strains were produced at will by growing the strains in liver infusion mediums. These cocci reverted to bacilli when transferred to favorable mediums. A direct microscopic observation on the warm stage was made of the bacillus-to-coccus and coccus-to-bacillus change. The virulence, toxigenesis and fermentation reactions of the coccus forms could not be satisfactorily determined. The differentiation of diphtheria and diphtheroid strains on liver infusion agar was not cleancut enough for diagnostic purposes. When methods employed by other workers for producing coccus forms were successfully duplicated, the resulting cocci were morphologically indistinguishable from those produced in liver infusion mediums. The addition of glycogen, dextrose or bile to veal infusion broth did not induce the formation of cocci.

FROM THE AUTHOR'S SUMMARY

THE PATHOGENICITY FOR CATTLE OF BRUCELLA STRAINS FROM UNDULANT FEVER IN MAN R R BIRCH and H L GHIMAN, J Infect Dis 56 78, 1935

Strains of Brucella isolated from patients with typical undulant fever proved regularly to be pathogenic for cattle, producing in them a syndrome indistinguishable from that which occurs in natural cases of Brucella infection otherwise known as infectious abortion.

FROM THE AUTHORS' CONCLUSION

THE SPREAD OF AMEBIC DYSENTERY BY WATER A V HARDY and B K SPECTOR, Pub Health Rep 50 323, 1935

During the stockyards fire in Chicago in May 1934 water heavily polluted with fresh sewage was used for drinking purposes by many firemen and spectators. There followed a large but undetermined number of cases of acute diarrhea, sixty-nine of typhoid fever and two of paratyphoid fever. Laboratory studies revealed

that a high percentage of those exposed had become infested with *Endamoeba histolytica*, and the more severe the symptoms, the higher the percentage. Six cases of undoubted amebic dysentery, two complicated with abscess of the liver, were recognized among those exposed. Six other mild diarrheas (one in a fireman and five in spectators) were also diagnosed as amebic dysentery and reported to the board of health. The evidence has led to the opinion that many of the other illnesses with intestinal symptoms were also the result of *E. histolytica* invasion.

Therefore it is concluded that infestations with *E. histolytica* may occur in association with water-borne epidemic diseases, and, furthermore, that the control of amebic dysentery demands that water for human consumption be free from dangerous protozoal as well as from bacterial contamination.

FROM THE AUTHORS' SUMMARY

THE EXPERIMENTAL PRODUCTION OF MUMPS IN MONKEYS G. M. FINDLAY and L. P. CLARKE, Brit J Exper Path **15** 309, 1934

Bacteriologically sterile saliva taken from a patient with mumps forty-eight hours after the onset and injected directly into Stensen's duct in rhesus monkeys produces a mild febrile reaction, swelling of the parotid glands and histologic changes very similar to those seen in human mumps. There is also a leukopenia. The virus has been passed through monkeys six times. It can be preserved in 50 per cent glycerin for at least five weeks. Injection of the virus into the tunica vaginalis of a monkey causes a nonsuppurative orchitis. Mice, rats and guinea-pigs inoculated intracerebrally did not present any symptoms.

AUTHORS' SUMMARY

THE EXPERIMENTAL B VIRUS DISEASE IN MACACUS RHEBUS MONKEYS A. B. SABIN, Brit J Exper Path **15** 321, 1934

The B virus is pathogenic for rhesus monkeys and has been transmitted through four generations by intracerebral inoculation. Intracerebral inoculation of the virus is usually fatal, and gives rise to various clinical syndromes. The most striking features of the disease following intravenous inoculation of the virus are an exanthem (chiefly about the forehead, eyes and face) and an enanthem (in the buccal mucous membranes, tongue, palate, conjunctivae) which closely resemble the picture produced by intravenous inoculation of vaccinia in rhesus monkeys. Intracutaneous injection of the virus is followed by acute inflammation with necrosis, which may be hemorrhagic or vesiculopustular. Macroscopically the lesion bears some resemblance to that produced by vaccinia, but microscopically it is readily differentiated from the latter by the presence of acidophilic intranuclear inclusions in the epithelial cells and by the absence of Guarnieri bodies. Intraperitoneal injection of the virus gives rise to acute peritonitis with occasional invasion of some of the abdominal viscera. In monkeys peripheral inoculation of the virus does not lead to any apparent involvement of the central nervous system, and in this respect the virus lacks one of its most striking and constant properties in rabbits. Among 13 "normal" monkey serums, only one was found to contain antibodies for the B virus, and the titer of this one was as high as that of the best hyperimmune serum, on this basis it is believed that the monkey may at one time have had a natural B virus infection. Similarly, the opinion is expressed that the reason for the failure to establish this virus in rhesus monkeys in New York is that the majority of them were immune probably as a result of natural infection, and that the human disease from which the B virus was isolated was caused by the bite of a monkey which was infected with it.

FROM THE AUTHOR'S SUMMARY

ENCEPHALOMYELITIS PRODUCED BY NEUROTROPIC YELLOW FEVER VIRUS G M FINDLAY and R O STERN, *J Path & Bact* **40** 311, 1935

The neurotropic yellow fever virus produced in susceptible animals (*a*) an inflammatory reaction in the central nervous system, (*b*) degenerative changes in the nerve cells and (*c*) acidophilic intranuclear inclusions. The inflammatory reaction was characterized by infiltration with mononuclear cells and proliferation of the microglia. Degenerative changes in the ganglion cells were present in all stages and ranged from slight swelling of the cell body to neuronophagia with complete disintegration of the cell. Specific acidophilic intranuclear inclusions were seen only in ganglion cells which had not undergone extensive degeneration and never in cells exhibiting neuronophagia. The inclusions did not stain with the Feulgen technic. Demyelination was nowhere observed.

FROM THE AUTHORS' CONCLUSIONS

DIFFERENTIATION OF STREPTOCOCCUS PYOGENES FROM MAN AND ANIMALS BY THE SORBITOL-TREHALOSE TEST F C MINETT, *J Path & Bact* **40** 357, 1935

Examination of a series of strains of *Str pyogenes* from man and from cow's milk has given support to the view that strains of this kind from man are trehalose fermenters, while those from cow's milk are ordinarily sorbitol fermenters. Considered in conjunction with the work of previous observers, the sorbitol-trehalose test thus appears to be a simple method of establishing the original source of *Str pyogenes* when present in milk. Apart from *Streptococcus equi*, ten of twelve strains isolated from equines fermented sorbitol and not trehalose. Judged by the methylthionine chloride reduction test, however, the two remaining strains were probably of animal type. Eleven of the twelve strains from dogs, cats and ferrets fermented neither sorbitol nor trehalose but differed from *Str equi* in fermenting lactose. Attention is again called to a group of streptococci found in milk which ferment both trehalose and sorbitol. These streptococci, however, although they are actively hemolytic and fail to split sodium hippurate, should be excluded from the *Str pyogenes* group.

FROM THE AUTHOR'S SUMMARY

Tumors

A REVIEW OF THE RECENT LITERATURE OF TAR CANCER (1927-1931 INCLUSIVE) M G SEELIG and Z K COOPER, *Am J Cancer* **17** 589, 1933

Our purpose, in this paper, is to bring, within convenient and compact form, the literature of tar cancer up to the beginning of 1932. William H Woglom (*ARCH PATH* **2** 533, 1926) collected and abstracted the literature of tar cancer from the epochal work of Yamagiwa and Itchikawa in 1914 down to the end of 1926. Woglom's work not only constitutes a practical lexicon for all workers in tar cancer, but also makes it imperative that the encyclopedic topic of tar cancer be brought down to date, however trying and uninspiring may be the task of collective abstracting.

AUTHORS' SUMMARY

MULTIPLE MYELOMA E SCOTT, F M STANTON and M OLIVER, *Am J Cancer* **17** 682, 1933

To the 425 cases of multiple myeloma already collected from the literature by Geschickter and Copeland, we have added 30 cases from the recent literature and 5 cases of plasma cell multiple myeloma occurring in our series of autopsies. From a study of maturation stages in fast-growing myelomas and their similarity to experimentally produced plasma cells, we consider it probable that the tumor plasma cell is a derivative of the reticular cell of the hematopoietic and general connective tissues and is closely related to the lymphocyte series.

AUTHORS' SUMMARY

GENETIC STUDIES ON THE TRANSPLANTATION OF TUMORS J J BITTNER, *Am J Cancer* **17** 699, 709, 717 and 724, 1933

The tumors inoculated in these experiments arose spontaneously and independently of each other in a single host. This mouse, no 19308, was an F_1 hybrid animal produced by mating individuals of two highly inbred stocks of mice, the albino and the dilute brown. All tumors were diagnosed as adenocarcinomas of the mammary gland. They were termed 19308 A, B and C and were inoculated simultaneously into individuals of the two parental stocks and their reciprocal F_1 , F_2 and back-cross generations. The total number of mice inoculated was 4,375.

Mice of the parental stocks gave negative results in grafts of tumor 19308 A, whereas in the F_1 generation 99.6 per cent of the animals inoculated were susceptible. The respective reciprocal hybrid generations gave differences which were not mathematically significant, the F_2 generation, observations which, when compared with expectations, showed that tumor 19308 A required the simultaneous presence of eight dominant mendelian factors for progressive growth, one of which was linked with the dilution color factor. Five of the susceptibility factors were derived from the dilute brown parental stock and three from the albino parental stock.

Tumor 19308 B grew progressively in all mice having the seven dominant mendelian factors necessary for susceptibility. One of the factors was linked with the dilution color factor. Three of the factors were contributed by the albino-race. The number derived from the dilute brown stock was between four and five. Two dilute brown males (litter-mates) grew tumor 19308 B.

Tumor 19308 C also required seven dominant factors for susceptibility, one of which was linked with the dilution color factor. Two or three of the susceptibility factors were derived from the albino stock and between four and five from the dilute brown stock.

Since tumors 19308 A, B and C were transplanted simultaneously, the observations were compared and an attempt was made to determine the number of susceptibility factors common to all three tumors. This number was probably five.

The value of the transplantation of neoplastic tissue still remains to be determined. It does show, however, that, on the simultaneous inoculation of multiple tumors from a single host, the physiologic and genetic constitution of some tumors is not identical with that of the host which gave rise to them.

Data secured from the transplantation of neoplastic tissue may aid in understanding the problem of cancer if one assumes that (1) the etiologic factors may be due to hereditary or environmental causes, (2) cancer, if inherited, may be explained according to multiple dominant factors, (3) the physiologic characteristics of each cancer are determined by its genetic constitution, and (4) multiple neoplasms from a single host may be the same histologically but differ genetically and physiologically.

AUTHOR'S SUMMARY

ON THE AMBIVALENT EFFECT OF A SINGLE ACUTE TRAUMA ON THE GROWTH OF TRANSPLANTED MOUSE TUMORS M C MARSH, *Am J Cancer* **17** 735, 1933

Sixty-three transplanted mouse tumors were crushed by one sudden pressure, and their subsequent growth was frequently measured. A sharp difference appeared between the reactions of smaller and larger tumors. The twenty-nine tumors that were 4 mm or less in diameter were not inhibited to a significant degree. The thirty-four tumors above 4 mm were stimulated in a degree that is probably statistically significant. When all increments of growth were combined and averaged the stimulation was nominal. There were seventy-one control tumors.

AUTHOR'S SUMMARY

EMBRYONAL NEPHROMA IN A SHEEP W H FELDMAN, *Am J Cancer* **17** 743, 1933

The occurrence of an embryonal type of renal tumor in an 8 months' old sheep is reported. The tumor, which is designated as an embryonal nephroma, had

metastasized to the sublumbar lymph nodes and to the lungs. Although this type of neoplasm is not uncommon in chickens and swine, and has been reported in rabbits and in cattle, it apparently has not been reported previously in sheep.

AUTHOR'S SUMMARY

ABSORPTION OF THE PROTECTIVE AGENT FROM RATS RESISTANT TO A TRANS-PLANTABLE SARCOMA. WILLIAM H WOGLOM, *Am J Cancer* **17** 873, 1933

Frozen and thawed rat sarcoma 39 which has lain for a few days in the subcutaneous tissues of an immune rat accumulates from them a substance which is not present in normal rats, and then exerts an inhibitory effect on the growth of this neoplasm when mixed with it prior to inoculation. As the cells of R 39 are killed by freezing and thawing, it may be fairly said that the suppressing agent in immune rats has been separated from the living cell, though not from living protoplasm.

AUTHOR'S SUMMARY

GENETIC FACTORS IN THE ETIOLOGY OF MALIGNANT TUMORS. M R CURTIS, W F DUNNING and F D BULLOCK, *Am J Cancer* **17** 894, 1933

Possibly when more is known about the etiology of other tumors (besides cysticercus sarcoma) for which there appears to be an inherited susceptibility, and when the expression of genetic factors in the cells and tissues is better understood, it will be found that in the case of all neoplasms, in all species, the initial cellular change occurs by a process analogous to somatic mutation, and that hereditary factors determine this change only so far as they influence longevity and the susceptibility of a person to some specific irritant or condition which is favorable to mutation.

AUTHORS' SUMMARY

THE POTASSIUM CONTENT OF BENIGN UTERINE TUMORS. L C SCOTT, *Am J Cancer* **17** 924, 1933

The means of potassium and ash were found to be 0.166 and 0.913 Gm per hundred grams of moist tissue in ninety-eight benign uterine tumors, while the mean of moisture was 79.58 Gm per hundred grams in ninety-seven tumors. The correlation coefficients were significant for potassium-ash and potassium-water, but not for water-ash. The mean, median and mode for potassium were the same for the ninety-eight tumors, taken collectively, and for forty-three tumors selected according to their macroscopic appearance and representing the usual type of tumor encountered. The potassium content of uterine fibromyomas is, in general, not in excess of that of normal tissue, in fact, the mean of ninety-eight tumors indicates that it is usually somewhat less.

AUTHOR'S SUMMARY

QUANTITATIVE BEHAVIOR OF PROLAN A IN TERATOMA TESTIS. R S FERGUSON, *Am J Cancer* **18** 269, 1933

A series of 117 consecutive cases of teratoma testis in which the quantitative excretion of prolan A has been studied is reported. The technic for the quantitative estimation of prolan A in the urine is described. The patient with teratoma testis will excrete from 50 to 50,000 mouse units of prolan A per liter of urine, depending on the embryonal character of the tumor, the extent of the disease and the status as regards treatment. Irradiation of the primary tumor and its metastases causes a decrease in the excretion of prolan A in the urine. Local recurrence or metastasis is accompanied by an increase in the excretion of prolan A in the urine, frequently before clinical detection of the lesion is possible. Serial observations at frequent intervals while the patient is under active treatment by irradiation give important prognostic information. Observations at autopsy reveal the important relation between the hormone of the anterior lobe of the pituitary and epithelial hyper-

plasia in the genital organs of the male, particularly the prostate and seminal vesicles. The significance of these observations is briefly discussed.

AUTHOR'S SUMMARY

THE RELATION OF HEREDITY TO CANCER OCCURRENCE AS SHOWN IN STRAIN 73
MAUD SLYE, *Am J Cancer* **18** 535, 1933

From consistent results obtained in this laboratory during twenty-three years and involving over 116,000 autopsies, the general trend indicates two facts clearly. 1. The genetic difference between susceptibility and immunity to cancer involves one gene, that is, they are unit characters. 2. In the stock in this laboratory susceptibility to cancer behaves like a recessive immunity, like a dominant.

AUTHOR'S SUMMARY

THE INFLUENCE OF HORMONES ON THE GROWTH OF CARCINOMA, SARCOMA,
AND MELANOMA IN ANIMALS. K. SUGIURA and S. R. BENEDICT, *Am J Cancer* **18** 583, 1933

An investigation has been made of the effect of insulin, thyroxin U. S. P., parathyroid extract, epinephrine, theelin, theelol, emmenin, aqueous and alcoholic extracts of calf thymus, aqueous, alcoholic, ether and glycerin extracts of suprarenal cortex (sheep and cattle) and aqueous and alcoholic extracts of beef pituitary gland on Flexner-Jobling rat carcinoma, Jensen rat sarcoma, Segura rat sarcoma, Gye mouse sarcoma 37, a transplantable mouse melanoma and Rous chicken sarcoma 1. The repeated subcutaneous or intramuscular injection of the hormones or the extracts of endocrine organs had no curative or retarding influence on the growth of transplanted tumors. Injection of aqueous extracts prepared from whole pituitary glands or from the anterior lobes had a slight but distinct stimulating effect on the growth of mouse melanoma and Segura rat sarcoma. In the other tumors mentioned in this paper no stimulating action was observed. None of the other endocrine organ extracts or hormone preparations exerted any demonstrable accelerating influence on tumor growth.

AUTHORS' SUMMARY

MULTIPLE MYELOMA. NORBERT ENZER and BENJAMIN LIEBERMAN, *Ann Int Med* **8** 1062, 1935

A case of multiple myeloma with features simulating hyperparathyroidism is reported. The possible role of the parathyroid glands in myelomatous and other generalized bone diseases is considered. In the case reported, atrophy of the parathyroid gland was encountered, and secondary or functional hyperparathyroidism is questioned. In both myelomatosis and hyperparathyroidism there is a disturbance of the calcium balance.

FROM THE AUTHORS' SUMMARY

THE EFFECT OF MALIGNANT TUMOURS ON THE HYPOPHYSIS. C. S. McEuen, H. Selye and D. L. Thomson, *Brit J Exper Path* **15** 221, 1934

In agreement with previous workers we have observed vacuolation and other histologic changes in the anterior lobe of the hypophysis in rats bearing transplantable tumors. Since, however, we have produced similar changes by intraperitoneal implantation of kidney tissue it is concluded that the changes produced by transplantable tumors are not necessarily related to the malignant growth as such but are at least partly due to decomposition of tissue in the necrotic centers of the tumors.

FROM THE AUTHORS' SUMMARY

NUCLEUS-PLASMA RELATION OF CEREBRAL GLIOMAS. K. SCHAFER, *Beitr z path Anat u z allg Path* **92** 199, 1933

After a brief discussion of the classification of cerebral gliomas offered by Bailey and Cushing, Schaffer describes and pictures giant nuclei seen in spongio-

blastomas Such nuclei indicate a nucleus-plasma relation altered in favor of the nucleus. This nucleus-plasma relation is evidence of extreme anaplasia or dedifferentiation and of neoplastic proliferation brought about by the abnormally active nucleus. Alteration of the nucleus-plasma relation in favor of the cytoplasm, the nucleus remaining passive, indicates a benign reaction of the glia to noxious agents of various kinds. In many glial tumors the nucleus-plasma relation may be altered in some cells in favor of the cytoplasm and in other cells in favor of the nucleus but without the formation of giant nuclei, cells with such a variable nucleus-plasma relation are termed dysgenetic.

O T SCHULTZ

VERTEBRAL CHORDOMA O SIMON, *Deutsche Ztschr f Chir* **241** 805, 1933

Vertebral chordoma is the rarest of the three types of chordoma, cranial, caudal and vertebral. Five cases of the vertebral type are reviewed from the literature, and one new case is reported. This occurred in a woman aged 57. The tumor grew from the anterior surfaces of the cervical vertebrae. The growth, which recurred after removal, was lobulated and divided by septums from the capsule. It was made up of small polygonal cells and larger cells with vacuolated plasma. Vertebral chordoma may arise from remains of the notochord within the vertebral body, it may grow intravertebrally or antevertebrally.

SKIN CARCINOMA IN A SCAR PROTECTED WITH INSULATING TAPE W BUNGELER, *Munchen med Wchnschr* **81** 1619, 1934

Within four months a squamous cell carcinoma developed in a scar (burn) on the back of the hand of a man aged 43 years. This rapid development is ascribed to the use of tape containing tar. By using benzine as a cleansing agent, the patient had satisfied the conditions for producing experimentally a tar carcinoma of the skin.

AUTHOR'S SUMMARY

A MESOGLIOMA OF THE BRAIN W K BELEZKY, *Virchows Arch f path Anat* **290** 450, 1933

In previously abstracted articles Belezky has presented the evidence that led him to conclude that the portion of the glia which is composed of oligodendroglia and Hortega cells is mesodermal in origin. This constituent of nervous tissue he terms "mesoglia" to distinguish it from the ectoglia of ectodermal origin. In this communication he describes the histology of a diffusely growing, infiltrative tumor of the left cerebral hemisphere of a youth aged 17 years. This tumor he believes to have arisen from the mesoglia, hence he terms it "a mesoglioma." He bases his opinion on the following characteristics, which indicate an origin from mesoglia: marked polymorphism of the cells, the presence of giant cells, the relationship of the tumor tissue to the numerous vessels of the tumor, the relation of the tumor cells to the walls of the vessels and the origin of those cells from the cells of the vessel wall, the presence of histiocytes, and the morphology and staining reactions of the tumor cells. In the classification of Bailey and Cushing the tumor would belong in the oligodendroglioma group, but Belezky disagrees with these and other authors that such tumors are of ectoglial origin.

O T SCHULTZ

INVOLVEMENT OF CRANIAL BONE IN MENINGIOMA S A BERNSTEIN, *Virchows Arch f path Anat* **290** 500, 1933

Bernstein presents the results of a microscopic study of the changes in cranial bone brought about by dural endothelioma or meningioma. The work was done in Erdheim's laboratory and is based on six tumors of the convex surface of the brain, five with and one without osseous involvement, and seven basal tumors, of which two were associated with involvement of bone. The malignant meningiomas consist of three parts, a subdural, a dural and an intra-osseous part. The

tumor invades the bone by the channels that carry the vessels, evoking a fibroplastic and osteoplastic reaction that precedes the invading tumor tissue. Tumors of the convex surface lead to thickening of the adjacent invaded bone by a laminated osteophytic layer, which may be present externally as well as internally. Tumor cells may disappear from the sclerotic and condensed bone. The blood vessels may be obliterated by the fibrotic process, resulting in necrosis of bone. Diffusely growing tumors may spread more widely and more rapidly in the bone than in the dura, and may penetrate the pericranium. The invading basal tumors caused sclerosis of bone but did not lead to thickening of the bones as did the tumors of the vault. In the basal tumors the invading tumor tissue itself formed bone. An appended note refers briefly to a benign meningioma that caused osteoporosis and absorption of the overlying bone.

O T SCHULTZ

CRANIAL CHORDOMA K G KLING, *Acta path et microbiol Scandinav*, supp 16, 1933, p 194

A 45 year old woman died of pulmonary tuberculosis complicated by vague neurologic symptoms, particularly muscular atrophy. At autopsy there was found a tumor the size of a goose egg in the right posterior cranial fossa. It was made up of physaliphores (Virchow). These cells, which were in various stages of development, were arranged in islands and lobules. The tumor was limited by a capsule and had apparently grown gradually and slowly.

JACOB KLEIN

MALIGNANT MIXED TUMOR OF THE UTERUS RAGNAR OLINDER, *Acta path et microbiol Scandinav*, supp 16, 1933, p 314

A 52 year old woman with menorrhagia was found to have a mixed tumor in the posterior wall of the corpus uteri. Microscopic examination showed the tumor to consist of a mixture of epithelial, sarcomatous and cartilaginous cells. Recurrence showed it to be malignant.

JACOB KLEIN

PAPILLARY SYRINGOCYSTADENOMATOUS NEVUS AND ITS RELATION TO MALIGNANCY O REUTERWALL, *Acta path et microbiol Scandinav*, supp 16, 1933, p 376

Four cases of this congenital type of cystadenomatous papillary tumor of the sweat glands are reported. The origin from sweat glands is determined only after microscopic examination. Malignant transformation of such tumors is of interest. There are groups of epithelial cells which have become separated during embryonic development and are often exposed to chronic irritation. In case 4 of this report biopsy showed an area of typical basal cell cancer with parakeratotic pearl formation. Another case is described from the literature.

JACOB KLEIN

CONGENITAL PARTLY MALIGNANT NEOPLASM OF THE SCIATIC NERVE WITH CONSECUTIVE ELEPHANTIASIS K GROTH, *Acta path et microbiol Scandinav* 11 44, 1934

In a new-born boy the entire right sciatic nerve from the origin to the ends of the branches was the site of a mixed tumor of mesodermal origin. The tumor contained cartilage and was in parts sarcomatous. In the intradural part of the nerve were three nodules consisting of convoluted nerve fibers and fibromatous tissue. The case shows that primary tumors may develop in the peripheral nerves from the connective tissue of the nerve without the involvement of the sheath of Schwann. Groth reviews sixty-six recorded cases of congenital sarcoma and finds no previous instance of primary congenital sarcoma in the peripheral nervous system. The cause of the elephantiasis, which was predominantly lipomatous, appears to have been a disturbance of the circulation of the extremity due to pressure on the common iliac vein by the tumor.

HETEROTYPICAL EPITHELIAL TUMORS OF THE SALIVARY GLANDS AARNO SNELLMAN, Arb a d path Inst d Univ Helsingfors 7 419, 1933

Eight cases of heterotypical tumors of the salivary glands are reported, with clinical and microscopic details. Measurements of nuclei and nucleoli tended to indicate that greater size of the nucleoli was associated with greater malignancy and cellular anaplasia. The microcentrum and the number of centrioles also reflected to some extent the degree of malignancy. The morphology and localization of the Golgi apparatus and the chondriosomes seemed in no way related to the degree of malignancy. The adenomatous structure, the cell function, the size of the nucleus and of the nucleoli and the morphology of the cells tend to place the growths in the same group as the mixed tumors.

JACOB KIEIN

Medicolegal Pathology

FATAL ETHYLENE DICHLORIDE POISONING W C HUEPER and C SMITH, Am J M Sc 189 778, 1935

Ethylene dichloride, known since 1795 and first used as an anesthetic, has recently come into widespread commercial use as a solvent and cleaning fluid. A patient who drank 2 ounces (59 cc) of this liquid died within twenty-two hours. Stupor, vomiting, diarrhea, cyanosis and subnormal temperature were the outstanding clinical symptoms. Death was apparently caused by circulatory failure. The ingested substance caused an extensive hemorrhagic colitis, nephrosis with marked tubular calcifications and generalized passive congestion of the internal organs besides multiple perivascular hemorrhages in the region of the cerebral basal ganglia.

FROM THE AUTHORS' SUMMARY

TRAUMATIC RUPTURE OF THE HEART AND INTRAPERICARDIAL STRUCTURES S Z HAWKES, Am J Surg 27 503, 1935

This is a report of a study of seventy cases of injury to the heart and great vessels within the pericardium occurring in 2,708 deaths due to accidents. Such injuries may occur without fractures or visible evidence of external injury to the chest. In four cases the cardiac injury alone was responsible for death, in the other 66 cases there were associated ruptures of the lungs, liver, spleen or a hollow viscus or fracture of the skull or spine.

MAN LEDERER

MEDICOLEGAL APPLICATIONS OF BLOOD-GROUPING, WITH SPECIAL REFERENCE TO THE AGGLUTINOGENS M AND N OF LANDSTEINER AND LEVINE A S WIENER, Canad M A J 32 393, 1935

Blood grouping is of proved value in medicolegal cases of disputed paternity. Its application is based on experimental investigations of families involving tens of thousands of persons. Its reliability has also been established in various European countries in more than ten thousand medicolegal cases in which it has been applied. In this country the method has thus far been applied only in a small number of cases, and its introduction as a routine procedure in all cases of disputed filiation is urged. Blood grouping has also proved useful in criminal cases in which stains of blood, semen or saliva were found.

FROM THE AUTHOR'S SUMMARY

RUPTURE OF THE DIAPHRAGM FOLLOWING SLIGHT TRAUMA K R TATTERSALL and E B HARVEY, Brit M J 1 879, 1935

A man was struck by a lorry. Four ribs were fractured, there were signs of left hemothorax and shortly after admission to the hospital signs of intra-abdominal disturbance. He died the next day. The autopsy showed a tear in the left side

of the diaphragm about 4 inches (10 cm) long through which the kidney had been forced, the spleen was ruptured in four places, and there were fractures of anterior fossae at the base of the skull. With all these severe injuries there were no external marks of violence on the body.

MAX LEDERER

RED COLORATION OF THE BODY IN BARBITURIC POISONING F DERVIEUX ET AL,
Ann de med lég **14** 443, 1934

Attention is called to the fact that in some cases of barbituric poisoning there may be a more or less widely spread rosy red coloration of the body after death.

THE CAUSE OF THE DEATHS ASSOCIATED WITH THE FOG IN THE VALLEY OF THE
MEUSE J FIRKET, Ann de med leg **15** 2, 1935

The illnesses and deaths attributed to the fog which collected over the valley of the Meuse in 1930 were associated with increased concentration of sulphurous anhydride in the atmosphere. In industrial cities greater concentrations of sulphurous anhydride are expelled into the atmosphere, but the gas is constantly driven away by numerous sources of heat, thus preventing the formation of a fog.

INTRAVASCULAR MIGRATION OF PROJECTILES E CURTILLET, J de chir **44** 715,
1934

A case is described of a bullet wound in the pit of the stomach followed by migration of the bullet into the left femoral artery, from which it was removed successfully some weeks afterward. The cases of intravascular migration of projectiles recorded in the literature are reviewed and classified.

PRECIPITATING ANTIHEMOGLOBIN SERUM IN THE DIAGNOSIS OF BLOOD STAINS
B BALOTTA, Boll d Soc ital di biol sper **9** 934, 1934

Good results were obtained with antihemoglobin precipitin serum, human or animal, in the diagnosis of blood stains. The reactions were specific. No precipitates were formed when the antihemoglobin serum was mixed with sperm, bile or other protein-containing substances free from hemoglobin. Spots several years old gave good reactions. Similar results have been obtained with hemoglobin precipitins in identification of blood by Hektoen and Schulhof (*J Infect Dis* **33** 224, 1923).

RELATION BETWEEN THE SUBGROUPS AND TRANSFUSION REACTIONS N BLINOV,
Deutsche Ztschr f Chir **243** 400, 1934

Blinov studied 550 cases from the standpoint of the subgroups of blood group A and blood group AB. The ratio of subgroup A_1 to subgroup A_2 was found to be 4:1, and of A_1B to A_2B , 13:1. There were no fatal reactions when A_1 blood was given to persons with A_2 blood, but stronger reactions were obtained, on the whole, than when blood of the homologous subgroup was used.

A S WIENER

TECHNIC OF DEMONSTRATING THE PROPERTIES M AND N H ELBEL, Deutsche
Ztschr f d ges gerichtl Med **24** 242, 1935

Elbel has adopted the method suggested by Lattes of absorbing the diluted immune rabbit serum with boiled erythrocytes when preparing specific testing fluids. This method, he thinks, has the advantage that it is possible to obtain colorless testing fluids. However, in my experience, it is possible to obtain practically colorless testing fluids even when fresh cells are used if the immune serums are properly inactivated before performing the absorption. Besides, if the testing fluid has a slight pink tinge, this in no way interferes with the tests, but rather

has the advantage that it serves to identify the testing fluid. And, as Elbel points out, boiled erythrocytes have lesser absorbing power than fresh blood, so that much more blood is needed, and the preparation of the testing fluids is more tedious since several absorptions are necessary.

A S WIENER

GROUP SPECIFIC SUBSTANCES IN FRESH HUMAN MILK AND IN MILK STAINS
G STRASSMANN and K REISFELD, *Deutsche Ztschr f d ges gerichtl Med*
24 330, 1935

Tests for the presence of iso-agglutinins in fresh human milk yielded the following results. In twelve of nineteen specimens from group O persons both iso-agglutinins were demonstrable, in one only the anti-A agglutinin, in one only the anti-B agglutinin. In five neither iso-agglutinin was demonstrable. Tests for the group-specific substances in milk stains gave the following results. With none of seventeen stains from group O persons was nonspecific absorption observed, and in twelve of thirteen stains from group A and group B persons the corresponding group-specific substance was demonstrable. In the case in which the group-specific substance could not be demonstrated, the stain was taken from a "nonsecretor".

A S WIENER

ERRORS IN TECHNIC IN FORENSIC BLOOD GROUPING TEST A LAUER, *Med Welt*
8 933, 1934

The adoption by the courts of the tests for M and N in cases of disputed parentage doubled the chances of proving nonpaternity by blood tests. Many men who had not been excluded by tests previously made for the four classic Landsteiner blood groups applied for the newer M and N test in order to take advantage of the additional chance of exonerating themselves that these tests offered. Lauer thus had the opportunity of checking some of the results obtained by previous workers, and he found that in as many as eight cases errors in technic had been made despite the relative simplicity of the ordinary blood grouping tests. In not one of the cases in which errors had been made was the person who made the tests a serologist. Furthermore, the tests had been made with commercial serum, adequate controls had not been used, and the results had not been properly checked by testing each unknown serum against known cells. Errors of this sort can serve only to shake the confidence of the courts in these valuable tests. Of course, the difficulty is not with the tests but with the persons who make the tests. It is therefore essential, especially with the more difficult tests for M and N, to permit only properly qualified persons to perform the tests in medicolegal cases.

A S WIENER

EMBOLIC TRANSMISSION OF A RUBBER CATHETER INTO THE HEART IN ABORTION
J BLAHA, *Zentralbl f Gynak* 59 746, 1935

A woman requested a medical examination because on the previous day she had attempted an abortion by means of a rubber catheter. There was a perforation of the cervix. Laparotomy was resorted to, but a catheter could not be found. Curettage disclosed that there had been an incomplete abortion, and it was assumed that the catheter had induced the abortion and had been expelled without the woman noticing it. Sepsis developed and the woman died on the seventh day. At necropsy the lower, rough end (evidently broken off) of the catheter, which was 23.5 cm long and 4 mm thick, was detected in the inferior vena cava near the diaphragm. From there the catheter extended through the right auricle, the superior vena cava and the innominate vein into the left common jugular vein, in which the upper, smooth end of the catheter was found several centimeters above the clavicle. A comparison of the conditions at necropsy with the clinical course indicates that, following perforation of the cervix, the catheter must have entered a wide vein of the uterine plexus, from which it slipped directly into the right

hypogastric vein and into the inferior vena cava. It may be deduced from a number of symptoms that the catheter reached the heart on the fifth day following its introduction into the uterus. At any rate the catheter must have been in the heart for about forty-eight hours without causing severe impairment of the heart action. The author reviewed the literature on foreign bodies in the heart and found that in the majority of cases the foreign body was a projectile. He thinks that the reported case is the first one in which such a large foreign body entered the heart by embolic transmission. He reviews cases from the literature in which a catheter disappeared following introduction into the uterus. In some cases the catheter was extracted from the uterus, in others it entered the retroperitoneal tissue and was later extracted from an abscess, in others it entered the preperitoneal tissue and caused an abscess in the anterior abdominal wall, in others it entered Douglas' pouch, and in a considerable number of cases it entered the free abdominal cavity and produced diffuse peritonitis.

EXAMINATION FOR ALCOHOL IN PERSONS INJURED IN ACCIDENTS J HINDMARSH
and P LINDE, *Acta chir Scandinav* 75 198, 1934

At a hospital in Stockholm persons injured by accidents and admitted to the surgical clinic in the course of twelve months were subjected to a special examination with a view to finding out whether there had been any consumption of alcohol. The clinical examination was at times difficult on account of the patients' injuries. It was supplemented, however, by determination of the alcohol in the blood by Widmark's micromethod. It seems to the authors that this method is well suited for clinical use. It is emphasized, however, that difficulties may be encountered in making the test with patients affected by shock. In the present material good agreement is shown to exist between the clinical diagnosis and the alcohol content of the blood. The influence of alcohol even in low concentrations has generally been recognized. Widmark's earlier data on this point are discussed. It is emphasized that in cases of severe injuries one must often refrain from forming a judgment of the victim's state of sobriety. Accidents under the influence of alcohol are of common occurrence in traffic. It has been shown that also in the case of accidents during work a considerable amount of alcohol has sometimes been consumed. On admission to the hospital 41 per cent of all males injured in accidents (283) had alcohol in the blood, and of these as many as two thirds had more than 1 part per thousand. Those injured in traffic (115) showed about the same rate. Accidents under the influence of alcohol are commonest among more elderly people. Furthermore accidents tend to accumulate over week-ends and in relation to bank holidays. It is stated as a noteworthy fact that more than half of those who have overturned in driving a car or a horse, or who have fallen out of vehicles, or who as pedestrians have been knocked down, have had alcohol in the blood. This supports the view that in traffic accidents it is not only the driver of the vehicle who should have his state of soberness tested but also the injured persons.

FROM THE AUTHORS' SUMMARY

RESULTS OBTAINED FROM MEDICOLEGAL APPLICATION OF THE MN AND FOUR GENE
THEORIES E WOLFF and B JONSSON, *Acta path et microbiol Scandinav*
12 131, 1935

This excellent analysis of the theories as to the heredity of the blood groups and the MN types is based on data accumulated from a study of 600 cases of disputed paternity. The blood specimens were sent from all parts of Sweden, and it was shown statistically that the material was homogeneous from the standpoint of the distribution of the agglutinogens. Among the 1,200 adults tested, 37.92 per cent were of group O, 36.92 per cent of subgroup A₁, 9.75 per cent of subgroup A₂, 10.33 per cent of group B, 3.92 per cent of subgroup A₁B and 1.17 per cent of subgroup A₂B. Furthermore, 36.08 per cent belonged to type M, 47 per cent to type MN, and 16.92 per cent to type N. Hence, $p + q + r = 1.00144 \pm 0.00400$,

and does not differ significantly from unity. This test combined with a special analysis of the data in groups of 50 persons each supports the Bernstein theory of heredity of the blood groups. Furthermore, $m + n = 1.01200 \pm 0.01443$, so that the data also support the theory of Landsteiner and Levine as to the heredity of the agglutinogens M and N. Additional special statistical tests add further support to these theories, and also Thomsen's theory of the heredity of subgroups of group A and group AB. With special regard to the agglutinogens M and N, 709 mothers with 732 children were examined, but not a single exception to the theory of Landsteiner and Levine was found. In all, 5,835 mothers with 7,759 children have been studied by various investigators thus far. The authors therefore believe that exclusions of paternity based on the MN theory should be considered on the same level as those based on Bernstein's theory. More caution is required when applying Thomsen's theory, since the subgroups have not been as thoroughly studied yet.

A. S. WIENER

INCOMPATIBILITY OF THE BLOOD OF THE SAME GROUP DEPENDING ON AGGLUTININ FOR HITHERTO UNKNOWN RECEPTORS. A. ZACHO, *Hospitaltid* **70** 225, 1935

On account of severe hemorrhage on the separation of the placenta, the patient was given transfusions of blood from three donors of the same blood type as herself. During the subsequent days there were signs of disintegration of red blood corpuscles. Six days later another transfusion of 100 cc was given. The patient died five hours later. The donors and the patient were all of type A₁, but direct cross-matching tests of the cells and serums of donors and recipient were not made. This was done later and it was found that the patient's serum agglutinated the red corpuscles of two of the donors. The agglutinin in question appears not to have been recognized before, it agglutinated the red corpuscles in about 60 per cent of persons with O and A types of blood.

Technical

PARAFFIN SECTION METHOD FOR THE DOPA REACTION. S. W. BECKER, L. L. PRAVER and H. THATCHER, *Arch. Dermat. & Syph.* **31** 190, 1935

The dopa reaction was performed en bloc by the simplified technic of Laidlaw and Blackberg. The tissue was then fixed in Bouin's solution, embedded in paraffin and cut into thin or into thick sections if such are desired for a study of dendritic melanoblasts in their entirety. The sections may be stained by many methods, the most valuable of which for cytologic study is the Masson trichrome (iron hematoxylin, acid fuchsinponceau de xyldine and aniline blue), for which very thin sections must be used. All the sections showed that the dopa reaction does not occur in palisade basal cells, and no transitional forms were found between the palisade basal cells and the melanoblasts. These observations support the conception of the presence of two types of cells in the epidermis, one of which has to do with the regulation of pigment function. This method should make possible a more intelligent study of pigmentary processes by the dopa reaction than has been possible by the frozen section method.

FROM THE AUTHORS' SUMMARY

A NEW METHOD OF PRESERVING NORMAL AND PATHOLOGIC BRAIN TISSUE. JOSHUA ROSETT, *Arch. Neurol. & Psychiat.* **32** 513, 1934

For the preservation of brain specimens for teaching or other purposes Rosett recommends a new method which is devoid of the objectionable features in the use of fluids such as formaldehyde or alcohol. The new method consists in infiltrating the tissues with the insoluble, infusible synthetic resinoids of the phenol series. Slabs of brain tissue of any thickness preserved in formaldehyde are first placed for about one week in a 40 per cent solution of formaldehyde and then transferred to the foregoing solution of resinoids for ten days or longer, depending on the

thickness of the slabs After this they are subjected to polymerization, ground, polished and in some instances developed (in a solution of sodium hydroxide and salicylic acid) Certain precautions must be taken in handling the solution of the resinoids, for the description of which the original article should be consulted

GEORGE B HASSIN

CEREBROSPINAL FLUID IN TUBERCULOUS MENINGITIS H HOUSTON MERRITT and FRANK FREMONT-SMITH, Arch Neurol & Psychiat **33** 516, 1935

From a study of 297 cerebrospinal fluids in 84 verified cases of tuberculous meningitis it was found that aside from an increase in the pressure and in the number of white cells (lymphocytes predominating) there was a decrease in sugar content below 45 mg per hundred cubic centimeters and of chlorides below 650 mg per hundred cubic centimeters In the early stages the results may not be typical The pressure and the cell count, for instance, may be normal, but the contents of sugar, chlorides and protein are usually abnormal No difficulties are encountered in differentiating the condition from pneumococcic, staphylococcic, streptococcic and other types of meningitis, but it may be difficult to differentiate the condition from syphilitic meningitis, especially in the absence of typical serologic reaction

G B HASSIN

A SIMPLE METHOD FOR STAINING THE NERVE TISSUES E H LOUGHLIN, Arch Neurol & Psychiat **33** 616, 1935

Loughlin describes a combined gold and silver staining method which can be used not only on fresh brain tissues but also on tissues that have been fixed in solutions of formaldehyde for years, or in other solutions (Zenker's fluid, for instance), or that have not been fixed until several days after necropsy Loughlin's modification contains the essential features of the methods of Cajal and Bielschowsky Frozen sections are preferable, from 15 to 18 microns thick, but paraffin and pyroxylin (celloidin) sections also give good results As a mordanting agent, Loughlin uses dilute (2 per cent) bromine water (instead of hydrobromic acid) to which the frozen sections are transferred after they have been rinsed in water and kept in 5 per cent ammonia over night For the details the original article should be consulted As the photomicrographs show, the method brings out clearly the ganglion and various glia cells, mature and embryonic, their processes and the fat granule bodies, but less clearly the microglia

G B HASSIN

THE LABORATORY DIAGNOSIS OF AMEBIASIS T B MAGATH, J A M A **103** 1061, 1934

The direct smear method is adequate in the hands of those properly trained in almost all cases but, if doubt exists, one should resort to fixed and stained preparations Whether one uses formed stools or those resulting from catharsis will depend on the individual problem Whichever is used, the limitations of the particular method must be clearly recognized

Culture methods should be used in laboratories qualified to identify amebas, but, for the usual routine, cultures are not necessary, provided the examiner thoroughly knows how to make proper direct examinations The cultural characteristics of various amebas growing in these cultures have yet to be clearly described, and a series of animal experiments to determine the types in a large series is greatly needed

Until the complement-fixation method is simplified, it is not suitable for routine tests

Seventy-five per cent of infestations with *Endamoeba histolytica* can be found by examining a single stool following catharsis with magnesium sulphate, whereas only a third of the infestations will be found by examining a single formed stool It will require from eight to ten formed stools to establish the same number of infestations as three stools following catharsis

FROM AUTHOR'S SUMMARY

Society Transactions

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Twenty-Second Annual Meeting, Hotel Statler, Detroit, April 10-13, 1935

S BURT WOLBACH, *President*

SHIELDS WARREN, *Secretary*

THE BONE MARROW IN PATIENTS WITH CIRRHOSIS OF THE LIVER RAPHAEL ISAACS, University of Michigan

In patients with cirrhosis of the liver, the number of red blood cells larger than normal is increased. In some patients the blood picture simulates that of pernicious anemia. The bone marrow of 6 patients having cirrhosis of the liver and anemia was studied with reference to the number of cells per cubic millimeter and its differential composition of the types of nucleated cells. It was found that the number of red blood cells in the megaloblast stage is significantly increased correlating with (a) the macrocytosis of the mature red blood cells and (b) the favorable therapeutic response to liver extract.

EFFECT OF PRESSOR REACTIONS ON BACTERIAL LOCALIZATION IN THE CENTRAL NERVOUS SYSTEM WILLIAM F PETERSEN and A J NEDZEL (by invitation) University of Illinois

At the last meeting of the society we presented evidence indicating that pressor episodes were of significance for the localization of bacteria on the cardiac valves and demonstrated in dogs the experimental production of endocarditis when staphylococci and streptococci were injected intravenously shortly after the maximum pressor effect from injections of pitressin.

We have extended our observations to the localization of bacteria in the central nervous system. We used single as well as repeated injections of pitressin and a solution of the diuretic-antidiuretic hormone of the hypophysis (betahypophamine) to bring about transient anoxemia of the central nervous system and therewith foci of anoxic stimulation where localization of bacteria might be enhanced.

In the control animals we depended wholly on the possible passage of the normal flora of the mucous membranes, in the experimental animals the mucous membrane of the throat was sprayed with cultures of *Staphylococcus* and *Bacillus megatherium*.

In the case of a single injection of pitressin the numbers of bacteria seen in the region of the hippocampus major and in the spinal cord were small, similar to the findings in the normal dog without any injection of pitressin. In dogs that received a series of injections we found considerably larger numbers of microorganisms, the bacteria often forming groups, there was also distinct evidence of pathologic change in the nervous tissue. These changes are now being studied.

The findings mentioned lead us to the conclusion that pressor effects, no matter how induced, may lead to the localization of bacteria and to tissue change in places where these pressor effects (vascular spasm) are particularly pronounced.

PERTUSSIS PRODUCTION OF AN INTERSTITIAL MONONUCLEAR PNEUMONIA WITH THE BORDET-GENGOU BACILLUS DOUGLAS H SPRUNT and (by invitation) DONALD S MARTIN and JARRETT E WILLIAMS, Duke University School of Medicine

The presence of an interstitial mononuclear pneumonia in a patient with pertussis led McCordock to suggest that a virus may be the primary etiologic agent in this

disease This point of view was based on his preceding work with Muckenfuss on the relationship between viruses and this type of pneumonia Rich has further substantiated this relationship

In recent experiments we showed that exotoxins are capable of producing interstitial mononuclear pneumonia when injected intratracheally into rabbits This work led us to try similar experiments with the Bordet-Gengou bacillus

Pure cultures of this bacillus were kept in phase I by being grown on the Bordet-Gengou medium containing 25 per cent human blood Twenty rabbits were inoculated intratracheally with a suspension of the cultures All the rabbits acquired a typical interstitial mononuclear pneumonia As a control, 5 rabbits were given injections of cultures of the rough form of the Bordet-Gengou bacillus, grown on a similar medium, without producing any changes in the lung

Before the experiment the nares of the rabbits were swabbed and the material cultured for *Bacterium leprosepticum* and *Bacterium bronchisepticum*, with negative results At necropsy material from the lungs was cultured for organisms other than the Bordet-Gengou bacillus, without success

Conclusion—The Bordet-Gengou bacillus can produce interstitial mononuclear pneumonia in rabbits

SUSCEPTIBILITY TO TOXIC AGENTS ALTERED BY VARIATIONS IN THE GLYCOGEN AND FAT CONTENT OF THE LIVER JESSIE L BOLMAN and FRANK C MANN, The Mayo Clinic

The composition of the liver changes throughout the day in relation to the amount and character of the food taken The effects of toxic agents such as alcohol, chloroform, carbon tetrachloride or tetrachlorethane appear more marked if these agents are administered when the glycogen content of the liver is low This may be noted by the systemic effects of the drugs and by the extent of the pathologic changes produced in the liver Diets rich in carbohydrates raise the amount of glycogen in the liver as much as five times that found with the usual mixed diet Diets containing excessive fat may alter the fat content of the liver to ten times the usual one Susceptibility to toxic agents is increased with fat feeding and decreased with carbohydrate feeding Attention is also called to the rapid changes in the composition and histologic picture of the liver produced by diet or by toxic agents

STUDY OF STANDARD BREEDS OF RABBITS IN RELATION TO A TRANSPLANTABLE MALIGNANT TUMOR ALBERT E CASEY, The Rockefeller Institute for Medical Research and the University of Virginia

In the laboratory of Dr Wade H Brown at the Rockefeller Institute for Medical Research sample rabbits from 15 standard breeds were propagated in pure line over a period of from three to ten years None of the breeding stocks were used for experimental purposes during this period, and they were kept in the same rooms under identical conditions of food, light, air, caging and attention More than 250 healthy young adult males of approximately the same age from the families representing the 15 standard breeds (English, American Blue, Beveren, Flemish, Havana, Rex, French Silver, Polish, Himalayan, Belgian, Dutch, Sable, Special Albino, Tan and New Zealand) were inoculated in large groups with the same dose and emulsion of the Brown-Pearce rabbit tumor This tumor had been carried in this laboratory for ten years through mongrel stocks purchased from outside dealers and consisting largely of Belgian, Flemish, Dutch and Beveren mixtures The tumor, therefore, was not adapted to the standard breeds used for the test Two months after the inoculations (intratesticular) the surviving animals were killed and complete autopsies done The amount of primary and of metastatic tumor was measured by water displacement, and the number of metastatic foci per animal computed It was found that the breeds varied from almost 100 per cent susceptibility on the part of the English, American Blue and Sable types to almost 100 per cent resistance to the tumor on the part of the Havana and Himalayan breeds Analyzed statistically, the variance between the breeds was significantly

greater than the variance within the breeds ($P = 0.01$ —) This difference was of statistical significance as regards the incidence of primary tumors, the incidence of metastases, the number of metastatic foci per animal, the total volume of metastatic tumor per animal, the actual mortality, the total mortality and the longevity after inoculation These seven criteria were found to be reliable indexes of various phases of malignant growth in earlier experiments with this tumor Of the 25 English animals used, 92 per cent acquired primary tumors, and 100 per cent of those in which primary tumors developed acquired metastases The total volume of metastatic tumor per animal was 94 cc, and the number of metastatic foci per animal was 17.5 The actual mortality was 76 per cent, the expected mortality 84 per cent, and the total longevity forty-four days This compared with an almost 100 per cent resistance on the part of the Havana breed Of the 18 animals in this breed 22 per cent acquired primary tumors Of the 4 in which primary tumors developed, 50 per cent (2) had metastases, the volume of metastatic tumor per animal was 11 cc, and the number of metastatic foci per animal was 3 The actual mortality was 0 per cent, the expected mortality 11 per cent, and the total longevity sixty days (all animals were living at the termination of the experiment) The English and the Havana breeds were at opposite extremes on almost every point of malignancy analyzed The differences between the 2 rabbit breeds with the transplantable tumor are in every respect comparable with the results found in mice and rats with spontaneous tumors by selective inbreeding This is, perhaps, the first study of numerous metastatic phases of malignancy in genetically different breeds of a mammal Since this tumor metastasizes in various portions of the body in every way comparable to human tumors, and actually kills the animal by metastatic involvement of vital organs, the results are perhaps more applicable to the situation found in connection with human tumors than are the results of previous genetic studies of spontaneous tumors or of studies on transplanted tumors of small rodents with very low metastatic rates Furthermore, the degrees of susceptibility to the tumor shown by the 15 breeds coincide fairly closely with their degrees of susceptibility to experimental syphilis, spontaneous rabbit pox and spontaneous pneumonia, and with hereditary variations in their size, blood formulas and breeding characteristics, with certain individual exceptions

ARE THERE LIMITS TO THE CAPACITY FOR IMMUNOLOGIC RESPONSES? RUBEN L. KAHN and (by invitation) ELIZABETH B. McDERMOTT, University of Michigan

This is a report of progress in studies in which it is aimed to determine the extent to which increasing the number of immunizing injections of a protein antigen in an animal proportionately increases the immunologic capacities of the animal The antitoxin-toxin method of measuring the capacity of the tissues of an immunized animal to combine with specific antigen *in vivo* was employed for the determination of the immunologic response Five groups of rabbits were immunized with horse serum by means of, respectively, one, two, three, four and five injections of 0.2 cc per kilogram of body weight, given at ten day intervals Each immunized rabbit was given 50 MLD of diphtheria toxin and simultaneously a designated amount of horse serum antitoxin for diphtheria, considered as specific antigen, intracutaneously, about 5 cm distant Differences in the capacity of the skin to "anchor" the antitoxin, thereby preventing its diffusion from the area of injection and its neutralization of the toxin, were indicated by differences in the number of units necessary to save the animals from death due to toxin Thus, control rabbits not immunized with horse serum required 20 units of antitoxin for protection against 50 MLD of toxin Rabbits that had received one immunizing injection of horse serum required 300 units of antitoxin and were not protected by 200 units Rabbits that had received two immunizing injections were protected by 1,000 units but not by 750 units Rabbits that had received three immunizing injections were protected by 2,000 units but not by 1,750 units As a result of four and five immunizing injections, respectively, 2,500 units was required to protect, 2,000 units having failed to protect Increasing the number of immunizing injections of horse serum

evidently did not result in a proportional increase in the capacity of the skin to combine with the horse serum antitoxin. Similar results were obtained with a series of rabbits that were given six immunizing injections. It was also observed that the increase in the number of injections did not result in a proportional increase in serum precipitins. These studies suggest that the capacity for immunologic response is manifested to a moderate degree as a result of a first immunizing injection of protein antigen, that this capacity rapidly increases after a second and a third injection, respectively, and that the increase is comparatively small after a fourth, and still smaller after subsequent injections, indicating, in turn, that there are limits to the capacity for immunologic responses.

EFFECTS OF LOCAL IMMUNIZATION OF THE UPPER RESPIRATORY TRACT OF RABBITS
AGAINST PNEUMOCOCCUS, TYPE I THEODORE E WALSH (by invitation) and
PAUL R CANNON, The University of Chicago

Rabbits were immunized intranasally with a formaldehydized vaccine of Pneumococcus, type I, and their resistance to intranasal instillation of living virulent pneumococci determined. Control animals were treated intranasally with a vaccine of paratyphoid bacilli, with 0.4 per cent solution of formaldehyde or with 1 per cent solution of tannic acid. The object was to ascertain to what extent local immunization of an important portal of entry may protect a susceptible animal against infection by the same route, and to determine whether such resistance is predominantly local or general, specific or nonspecific. The results may be summarized as follows:

Eighteen of 20 normal animals infected intranasally with a saline suspension of living cultures of Pneumococcus, type I, died of a pneumococcic septicemia within from two to three days.

None of the 17 rabbits which had been immunized intranasally for from five to nine days with the formaldehydized pneumococcus vaccine died after intranasal infection with living cultures of this organism.

Eleven of 12 rabbits treated by intranasal instillation of a vaccine of paratyphoid bacilli, 0.4 per cent solution of formaldehyde or 1 per cent solution of tannic acid succumbed to the intranasal application of living cultures of Pneumococcus, type I.

We conclude from these experiments that a susceptible animal can be immunized intranasally by a specific bacterial vaccine so that subsequent intranasal infection leads to no serious invasion of the blood stream. Nonspecific stimulation of the tissues of the upper respiratory tract apparently confers no protection under similar conditions. Further experiments are in progress in an attempt to determine to what extent the increased resistance depends on local or on general processes of immunity.

FURTHER OBSERVATIONS ON THE CELLULAR CONSTITUENTS OF BOVINE SYNOVIAL
FLUID GRANVILLE A BENNETT and (by invitation) CHARLES F WARREN
and WALTER BAUER, Harvard University Medical School and Massachusetts
General Hospital

Under varying conditions, cytologic examinations have been made on specimens of synovial fluid from the astragalotibial and carpometacarpal joints of cattle and from the knee joints of dogs. From the data tabulated it has been possible to correct an error made in this laboratory concerning the classification of certain nucleated cells. The results may be summarized as follows:

When one compares specimens of synovial fluid from the astragalotibial and carpometacarpal joints of young beef cattle one observes wide variations in the total numbers of nucleated cells and in the percentages of the individual cell types. The greatest variation in the percentages of the cell types is noted in astragalotibial joint fluid.

The total numbers of nucleated cells and the percentages of phagocytic cells are greater in the carpometacarpal joint fluid than in the fluid from the astragalotibial

joint This difference is best explained by the increased amount of debris in the carpometacarpal joint resulting from the presence of defects in the articular cartilage

The wide variations in the numbers of the individual cell types observed in the astragalotibial joint fluid are evidently within the limits of the so-called normal The normal figure depends on the degree of wear and tear and minor traumas to which the joint has recently been subjected Evidently irritations of this grade are sufficient to increase the total number of nucleated cells and the percentage of phagocytic cells exclusive of polymorphonuclear leukocytes

The total number of nucleated cells contained in synovial fluid may increase post mortem, but there is very little change in the percentages of the individual cell types

Reexaminations of specimens of synovial fluid from two and one-half to three hours after the first count was made invariably revealed a marked increase in the number of dead or degenerating cells, together with considerable clumping Although the relative percentages of phagocytic cells were not materially altered, it became increasingly difficult to recognize distinguishing characteristics of the phagocytic cells

Several methods of study failed to reveal any evidence to support a theory that the cytology of synovial fluid is a reflection of that of the blood

A definite species difference in the total numbers of nucleated cells and the percentages of individual cell types contained in normal synovial fluid has been pointed out

The tabulated results obtained from this study should enable one to interpret more accurately the cytologic variations in normal and pathologic human synovial fluid

CHANGES IN THE PITUITARY GLAND FOLLOWING THYROIDECTOMY IN RATS ISORDE T ZICKWIR, University of Pennsylvania

The pituitary glands of white rats thyroidectomized when immature showed at fourteen days following operation an increase in the number of basophils and a decrease in that of acidophils, at thirty days, an almost complete disappearance of acidophils and an accumulation within basophils of large globules of smooth hyaline material, and as time increased up to two hundred and ninety days, condensation of the hyaline material into single globules within basophils, which caused these to simulate "castration cells" and which looked like increased production and storage of secretion It is suggested that the dwarfing of cretins is due to loss of acidophils Fat stains revealed no fatty degeneration Acidophils appeared to disappear by loss of granules and dedifferentiation rather than by degeneration Implantation of pituitary glands into guinea-pigs revealed an abundance of thyrotropic hormone in the pituitary glands of cretins, and it is likely that the amount of hormone available per gram of body weight of the cretin dwarf is greater than that of controls Thyrotropic hormone cannot have its source in acidophils It is suggested that basophils may produce thyrotropic hormone Male cretins showed large adrenal glands and testes in relation to body weight This suggests that there may be alterations in the proportions of adrenotropic and gonadotropic hormones coincident with the altered proportions of histologic types of cells

PATHOLOGIC CHANGES RESULTING FROM ACCURATELY CONTROLLED ARTIFICIAL FEVER F W HARTMAN and (by invitation) R C MAJOR, Henry Ford Hospital

The character of the lesions produced in the adrenal glands and brain were studied in detail as a basis for explaining the vascular collapse and cerebral symptoms that are noted following administration of artificial fever both in man and in experimental animals

EFFECT OF ARTIFICIAL FEVER ON ACUTE TUBERCULOSIS IN EXPERIMENTAL ANIMALS
R C MAJOR and H P DOUB (introduced by F W HARTMAN, Henry Ford Hospital)

Acute tuberculosis has been produced in dogs and monkeys, and the technique particularly of producing tuberculosis in the lung is described. The progress of the pulmonary lesion has been followed with roentgenograms, temperature charts and smears for tubercle bacilli. The animals were exposed to artificial fever in the Simpson-Kettering cabinet for periods of from four to five hours at a temperature of from 104 to 107 F at weekly intervals. This type of artificial fever does not seem to be harmful, and many of the lesions are affected favorably, as indicated by a disappearance of bacilli from the sputum, a return to normal temperature and a disappearance of the roentgen evidence of lesions.

DETERMINATION OF BODY SURFACE AREA BY PHOTOGRAPHY JOHN C BUGHNER and DAVID H DRUMMOND (introduced by CARL V WILKER), University of Michigan

Two methods are presented. In the first, the surface area is determined by mechanical quadrature, the body being assumed to be divided into approximately 100 cross-sections of uniform thickness. The perimeters of these elements are computed by considering the cross-sections as ellipses whose major and minor axes are the corresponding dimensions of the actual sections. The skin surface of each section is obtained by multiplying the perimeter by the average slant height. The summation of these elemental areas gives the total body area. The diameters are obtained from frontal and lateral photographic silhouettes. The probable error is approximately 2.5 per cent.

In the second method, which is a further development of the first, the measurements necessary are (1) the areas of the two silhouettes by planimeter, (2) the perimeters of the silhouettes by chartometer and (3) the height. By means of constants, the surface area may be quickly calculated. This method is slightly less accurate than the first but is more easily employed.

Both methods are independent of variations in body contour, so that the deviations encountered in the application of height-weight formulas do not occur.

THERAPEUTIC AND PROPHYLACTIC LIMITATIONS OF POLIOMYELITIS IMMUNE SERUM
E W SCHULTZ and (by invitation) L P GIBBIARDT, Stanford University

Twenty-four experiments were carried out in an effort to determine the therapeutic and prophylactic value of poliomyelitis immune serum. An immune horse serum of high virucidal titer was employed. This was administered by different routes and for the most part in large doses. Thirty-six monkeys received serum one or more days after inoculation with virus, and 91 monkeys received serum one or more days before inoculation. No therapeutic effect could be demonstrated in the former group, and less than a fourth of the animals in the latter group escaped the disease. Well defined protection was obtained only against small doses of virus.

The results are explained on the basis of recent observations which indicate that the virus is not only a highly neurotropic but probably an intracellular parasite. Once it becomes established in the olfactory nerve, it no longer can be reached by immune serum. Used prophylactically, serum has the effect of diminishing the amount of virus able to initiate infection. This is evidenced by a longer average incubation period in the serum-treated group. However, a dose of virus just sufficient to infect eventually leads to as extensive paralysis as is found in controls.

EXPERIMENTAL STUDIES DEMONSTRATING A RECIPROCAL RELATIONSHIP EXISTING BETWEEN LYMPHOPOIESIS AND MYELOPOIESIS B K WISEMAN, C A DOAN and (by invitation) L A ERF, Ohio State University

The introduction of simple proteins and of nucleic acid derivatives into the blood streams of two separate groups of rabbits resulted in characteristic changes

in the structures of the lymphatic and myeloid tissues. These alterations were reflected in a disturbance of the proportionate distribution of the cells in the circulating blood. Those animals receiving native protein (egg albumin, horse serum, etc.) showed a specific rise in the absolute numbers of lymphocytes accompanied by a striking decrease in the numbers of circulating neutrophilic leukocytes. Examination of the lymphatic and myeloid tissues during the period in which this disturbed relationship was prominent revealed hyperplastic changes in the former, associated with a striking hypoplasia in the latter.

In contrast to this reaction, the animals receiving nucleoprotein derivatives (nucleic acid, nucleotides) exhibited a marked neutrophilic leukocytosis with absolute lymphopenia. The tissue changes consisted of increased myelopoiesis (hyperplastic bone marrow and ectopic foci of granulopoiesis) associated with widespread degeneration of lymph structure (atrophy of lymphoid follicles and cyst formation).

Similar reciprocal changes in the tissues and blood of clinical patients showing abnormal lymphoid or myeloid stimulation have been observed and studied, and a correlation made with the changes observed in the experimental animals. These reciprocal hypoplastic and hyperplastic changes in the myeloid and lymphatic tissues tend to establish further the existence of a close relationship between the respective functional capacities of the hemopoietic organs, and they have important practical and theoretical implications that are of basic importance in the interpretation of the physiology and pathology of blood formation.

A STUDY OF BILE FROM THE HUMAN GALLBLADDER. CECILIA RIEGEL, I. S. RAVDIN and P. J. MORRISON, University of Pennsylvania

Bile Obtained from Patients with Noncalculus Cholecystitis—The bile removed at operation from patients whose condition was diagnosed as noncalculus disease of the gallbladder has been studied, and the results have been compared with those obtained on bile from the normal human gallbladder. The cases were separated into two groups—visualized and nonvisualized—according to whether the gallbladder was or was not visualized after the administration of sodium tetraiodophenolphthalein.

In both the visualized and the nonvisualized group the concentrations of chloride and bile salt were different from normal, the chloride being higher, the bile salt lower. No marked difference was found between the two groups in any of the various normal constituents studied. There was a tendency for the cholesterol concentrations to be somewhat higher in the nonvisualized group, but this was by no means constant. It was also true that the lowest calcium concentrations were found in the nonvisualized group.

The data are interesting in that they may show some of the initial changes which precede cholelithiasis.

Bile from Patients with Calculus Cholecystitis—We have carried out chemical analyses of the bile or stones or both taken from the gallbladder at the time of operation in 75 cases of calculus disease of the gallbladder. Cholecystographic reports were available in many of these cases. The cases have been classified into two groups—visualized and nonvisualized—according to whether the shadow of the gallbladder was or was not observed. The groups were further subdivided as to whether the stones were visualized as a positive or as a negative shadow. The data show a lack of correlation between visualization of the stones and chemical composition of the stones, the calcium content of the visualized stones was no higher than that of the nonvisualized ones. With two exceptions the stones contained chiefly cholesterol with varying amounts of calcium, pigment, phosphate, carbonate, organic debris, etc.

Analysis of the bile taken from the gallbladder at operation brought out several interesting points. In all cases the chemical composition of the bile was markedly altered from the normal. The chloride content was higher, the bile salt lower. In the visualized group the chloride content was as a rule lower, and the bile salt and cholesterol content higher, than in the nonvisualized group. In both groups the bile salt-cholesterol quotient tended to be lower than normal.

These experiments, taken in conjunction with previous work on normal and damaged gallbladders of dogs, on the composition of bile from human gallbladders with noncalculous cholecystitis, and on that of human hepatic bile after damage to the liver, indicate that in the final production of gallstones many varied factors may be involved

Bile from Pregnant Women at Term—Thirty-five specimens of bile obtained from pregnant women were analyzed for their concentrations of calcium, chloride, bile salt, cholesterol and phosphate. The data are significant in that they show in the majority of instances consistent change in the ability of the gallbladder membrane to concentrate bile. The concentrations of chloride, calcium and bile salt are as a rule similar to the concentrations of these substances in the gallbladder in cases of chronic cholecystitis. The cholesterol concentrations are, as a rule, considerably higher than those found in the bile from the normal gallbladder or in that from the chronically diseased gallbladder.

ABSENCE OF CHEMOTROPISM OF LYMPHOCYTES. HAROLD M. DIXON (by invitation) and MORTON McCUTCHEON, University of Pennsylvania

It is not understood how lymphocytes are accumulated in exudate, inflamed tissue and tubercles. Since one possible explanation is that these cells are attracted chemotropically, experiments were made to obtain evidence as to whether lymphocytes are capable of chemotropic reaction. Minute clumps (about 50 microns in diameter) of tubercle bacilli or of staphylococci were placed on a glass slide. A drop of blood from the finger was superimposed and allowed to spread between the slide and coverslip. With the microscope and a drawing ocular, the path of each lymphocyte and polymorphonuclear leukocyte was recorded on paper. For each white blood cell were determined (1) the net approach to the clump of bacteria and (2) the total path traversed in the same length of time. The ratio of these quantities is taken as the measure of chemotropism, +1 representing maximal positive, -1 maximal negative, chemotropism, and zero representing random movement or absence of chemotropism. When *Staph. albus* was used as the source of attraction, 60 polymorphonuclears showed strong positive chemotropism yielding a mean value of $+0.65$, in the same microscopic fields, the mean and the standard deviation for 45 lymphocytes were 0 ± 0.43 . With tubercle bacilli, the figure for polymorphonuclears was $+0.58$, for lymphocytes, -0.10 ± 0.43 . Hence, no evidence was obtained that lymphocytes are capable of chemotropism.

MORPHOLOGIC CHANGES IN THE CELLS FORMING EPIDERMOID CARCINOMA. JAMES E. DAVIS (introduced by F. W. HARTMAN), Wayne University

The material available for this study was obtained from tissues taken from the cervix uteri for biopsy which illustrated various pathologic changes, including over 200 examples of carcinomatous changes of the epidermoid type. Various interesting phenomena have been observed in following the gradations of changes in areas where natural repair was occurring. In the early stage of this repair the most immature or basal cell types were quantitatively increased in the complete layer of cells forming the protecting epithelial stratum. With each succeeding repair the quantity of immature cells was increased, and where irritation and disarrangement of the natural relations of the cells prevailed, cell maturation showed still greater delay. In the different stages of the malignant changes the quantity of immature cells varied until the ultimate picture showed that all of the cells in the stratified epithelial layer produced a heterogeneous arrangement of the three different layers without any suggestion of the original differentiation of basal, middle and superficial strata.

THE SERUM PHOSPHATASE IN COMPLETELY DEPANCREATIZED DOGS. VICTOR SCHELLING (introduced by F. W. HARTMAN), Henry Ford Hospital

The simultaneous decrease of inorganic phosphorus when sugar is removed from the blood has been interpreted as an intermediate compound of phosphorus

and hexose. In diabetic persons this fall is markedly delayed or even absent. As the formation of hexosephosphoric esters is presumably enzymatic, the serum phosphatase with an optimum pH 8.6 (Bodansky) has been studied in completely depancreatized dogs. In normal animals kept on a standard diet (Cowgill) for a long period the serum phosphate in the postabsorptive state fluctuates from 1 to 2 units (Bodansky) at different times. After complete removal of the pancreas the serum phosphatase increases from 20 to 30 units. Insulin produced no greater changes than were observed as limits of fluctuation in normal animals. Intravenously injected dextrose had no significant effect on the serum phosphatase determined in blood removed at definite intervals. It is concluded that if any formation of hexosephosphoric esters takes place in the blood of completely depancreatized dogs under the influence of insulin, serum phosphatase with the optimum pH 8.6 has no part in the reaction.

QUANTITATIVE STUDIES ON BLOOD COAGULATION. H. P. SMITH and (by invitation) E. D. WARNER and K. M. BRINKHUIS, University of Iowa

Quantitative methods for the titration of prothrombin and antithrombin in plasma are outlined. In such titrations one must distinguish between the titer of the actual antithrombin and the titer of the total amount which can be developed in the plasma with the aid of heparin. It is shown that with peptone injected into the circulation of the dog the antithrombic activity of the plasma rises, but not the titer of the potential total. The prothrombin content is normal. An injection of India ink has much the same effect as an injection of peptone. Chloroform poisoning causes a profound fall in prothrombin and fibrinogen, together with a moderate fall in platelets and in antithrombin.

EFFECT OF TOTAL HEPATECTOMY ON THE BLOOD FATS. STEPHEN MADDOCK, Boston City Hospital

In a series of dogs the blood fats were studied following total removal of the liver. It was found that a diminution in all of the constituents of the blood fat occurred in every instance. This change becomes appreciable at the twelfth hour and appears to be progressive throughout the remainder of the animal's life.

REFLECTION OF METEOROLOGICAL INFLUENCES IN THE SERUM REACTIONS (WASSERMANN, KAHN, ETC.). WILLIAM F. PETERSEN and (by invitation) E. T. HOVERSON and A. J. GOLDSTEIN, University of Illinois

Pressor episodes occurring in man are predominantly meteorologically induced. With pressor episodes reorientation (autonomic, biochemical, etc.) of the entire organism follows, with somatic stimulation evident in many ways.

In view of the distinct environmental effect that is clinically apparent in syphilis, including neurosyphilis, it seemed possible that the serologic reactions might reflect alterations of the meteorological status, and studies were carried out to determine this point for both the Wassermann and the Kahn reactions.

When daily titrations were carried out in patients with dementia paralytica it was found that the titers of the Wassermann and Kahn reactions varied from day to day with a distinct increase occurring in the wake of pressor episodes (usually occurring with polar infalls). These effects may be cumulative. In this behavior the serum reactions merely resemble or reflect somatic changes which are closely interwoven with the meteorological environment.

ETIOLOGY OF AMYLOID DISEASE. HOBART A. REIMANN and (by invitation) CARL M. EKLUND, University of Minnesota

Several theories have been advanced to account for the formation and deposition of amyloid substance: (1) that it is due to a general disturbance in the metabolism of protein, (2) that it represents an antigen-antibody union, (3) that it manifests disturbance or abnormality of the reticulo-endothelial system, (4)

that it is a feature of hyperglobulinemia, and (5) that it presents a combination of the conditions named in 3 and 4

Theoretical considerations lead us to believe that a long-standing increase of normal or abnormal blood globulins is one of the chief factors since (a) hyperglobulinemia is present in many cases of long-standing infection, malignant tumor and myeloma, conditions frequently preceding amyloidosis, (b) there is an increase of blood globulins in the patients with amyloidosis, (c) amyloid disease may be produced experimentally by excessive feeding with protein or by repeated injections of vaccine, which are known to cause hyperglobulinemia

Four rabbits were given about ninety intramuscular injections of sodium caseinate over a period of from eight to thirteen months. The amount of blood globulin was elevated after the first few injections and thereafter remained abnormally high (from two to four times over the normal level) until death. Early in the course the total amount of the blood protein was increased, but toward the end when evidence of renal amyloidosis appeared, the level was subnormal. All the animals died, and at necropsy extensive amyloid disease was found. The greatest amount of amyloid substance was found in the kidneys, less was present in the liver and spleen

VARIATIONS IN THE CATAPHORETIC MOBILITIES OF STREPTOCOCCI ISOLATED WEEKLY FOR ONE YEAR FROM THE NASOPHARYNGES OF TWO GROUPS OF WHITE PERSONS, FROM RAW MILK AND FROM LABORATORY STRAINS. EDWARD C. ROSENOW and (by invitation) CAROL B. PRATT and CHARLES SHIARD, the Mayo Foundation

The paper reports a parallel study of streptococci isolated weekly for one year from (1) the nasopharynges of a group of nurses, (2) the nasopharynges of a group of laboratory workers, (3) raw milk, (4) strains maintained in the laboratory. The frequency distribution curves of cataphoretic mobility were so grouped as to be attributable to a cycle of changes in the electrophoretic characteristics of the bacteria in each group. Parallel changes in the streptococci from the four groups seemed to be correlated with certain seasonal epidemic respiratory infections

TRANSMISSION OF EQUINE ENCEPHALOMYELITIS BY *Aedes Aegypti*. CARL TEN BROECK and (by invitation) MARCO V. H. MIRRELI, the Rockefeller Institute for Medical Research

Kelser's demonstration that the western strain of equine encephalomyelitis virus may be transmitted by *Aedes aegypti* gives us for the first time an insect-borne virus disease of animals that may be handled with ease in the laboratory. Our attempts to confirm his findings have brought out the fact that the mosquitoes must be fed a large amount of virus before they become infective. When they are fed small amounts, the virus can be demonstrated immediately, but it is lost in the course of a few days. We have also found that the western strain of encephalomyelitis virus will increase in the body of *A. aegypti*. It has been carried through a series of fifteen lots of mosquitoes by feeding normal mosquitoes on the crushed bodies of infected ones, and the virus has reached a dilution far beyond that which could infect if increase had not taken place. Another fact that has come out is that while *A. aegypti* will readily transmit the western strain of virus, it rarely is able to transmit the eastern strain. We have never succeeded in getting mosquitoes fed on suspensions of the brains of guinea-pigs infected with the eastern strain to transmit the virus by biting, while those fed on infected guinea-pigs occasionally are able to transmit it.

PROTEOLYTIC ENZYMES OF MONOCYCLIC AND POLYMORPHONUCLEAR PLEURAL EXUDATES. CHARLES WEISS and (by invitation) E. J. CZARNETZKY, Mount Zion Hospital, San Francisco

This article will be published in full in a subsequent issue of the ARCHIVES

PHILADELPHIA PATHOLOGICAL SOCIETY

*Regular Meeting, Feb 14 1935*MORTON McCUTCHEON, *President, in the Chair*

PARAGANGLIOMA (?) OF THE ADRENAL MEDULLA JOHN EGOROVICH

A markedly emaciated white Englishman of 59 years had complained of increasing weakness for a year. There were several episodes of vertigo with falls and injury. One week before admission to the hospital he had visual hallucinations. Four years before death his blood pressure was 150 systolic and 60 diastolic, and about six hours before death it was 140 systolic and 120 diastolic. Death was attributed to cardiac failure.

Necropsy revealed severe congestion of all the organs with areas of degeneration in the right diencephalon and both mamillary bodies. The heart weighed 210 Gm and was soft and dull brown. The kidneys were involved by benign nephrosclerosis. The left adrenal gland contained a circumscribed, spherical, gray-brown medullary tumor, 1.5 cm in diameter, surrounded by a thin layer of cortex, the cut surface was studded with deep red pinpoint dots. The opposite adrenal gland was normal. Secondary tumors were not seen.

In histologic sections the tumors were unencapsulated and consisted mainly of large polyhedral cells having a palely staining cytoplasm and vesicular nuclei. Large numbers of the cells contained dustlike pigment, which gave the specific green chromaffin stain with Giemsa's method. Some areas showed a dense admixture of small round, oval or spindle-shaped cells, the last having long polar processes which extended along the alveolar septums. Throughout the mass extensive hemorrhage and necrosis had occurred with deposits of hemosiderin, accompanied by lymphocyte and plasmocyte infiltration. Large cavernous spaces filled with a clear pink staining fluid (lymph) formed the center. The stroma was finely fibrillar.

The relation of the tumor to the hypertension is on purely speculative ground. The heart was small and not of the hypertensive type, it is suggested that the hypertension may have been paroxysmal, possibly accounting for the episodes of vertigo.

NEUROBLASTOMA OF THE ADRENAL GLAND WITH HEPATIC METASTASIS SIMULATING VON GIERKE'S DISEASE EDWARD S THORPE JR

An infant of 3 months was admitted to the pediatric service of the Philadelphia General Hospital on Sept 9, 1934, presenting chiefly enlargement of the abdomen and constipation. Its birth had been normal, and its development up to a few weeks before admission to the hospital had been uneventful. The only physical findings of note were a moderate secondary anemia, a leukocytosis of 21,000, of which 87 per cent were lymphocytes, and enlargement of the liver. The liver was so large that the mass filled both flanks and extended below the symphysis pubis. In the belief that the diagnosis was glycogenic hepatomegalia (von Gierke) the condition was studied along the lines indicated by van Creveld. The child showed a constant hypoglycemia which was affected very little by the injection of epinephrine, a constant ketonuria which showed a paradoxical reaction following the injection of epinephrine, a slight increase in the blood glycogen and a negative reaction to the Takata-Ara test for hepatic function. Twelve days after admission of the baby to the hospital, the temperature rose abruptly and it died, apparently with edema of the lungs.

Significant observations at necropsy were a tumor, 2.3 by 3 by 3.5 cm, in the left adrenal medulla, a tumor 2 mm in diameter in the right adrenal medulla, and almost complete replacement of hepatic tissue by a secondary tumor, the liver weighing 1,230 Gm. The primary adrenal tumors were well circumscribed and sharply demarcated from the overlying cortex, the tumor tissue being mottled

dark red to red-gray and trabeculated with fine strands of fibrous tissue. Histologically the tumor was composed almost exclusively of circlets of small hyperchromatic lymphocyte-like cells ("rosettes") with a fine pink-staining central tuft, the intervening stroma was hemorrhagic, and large areas of necrosis were prominent. The behavior of the tumor, i. e., metastasizing exclusively to the liver, characterized it as the so-called Pepper type of neuroblastoma.

NEUROBLASTOMA OF THE ADRENAL MEDULLA (HUTCHISON'S TYPE) D. R. COMAN

A colored girl, aged 2 years, was admitted to the hospital Sept. 22, 1934, with the history that three weeks previously she had been taken ill with malaise, fever, vomiting, a loss of weight and constipation. Her temperature on admission was 101.2 F. Physical examination revealed a rough systolic murmur, a prominent abdomen, an umbilical hernia, a palpable liver, a large abdominal mass and 2,600,000 red blood cells per cubic millimeter with 38 per cent hemoglobin. The child failed rather rapidly and died on October 5.

The clinical diagnosis was retroperitoneal tumor.

At autopsy the abdominal cavity contained about 500 cc of bloody fluid, and the retroperitoneum showed scattered punctate hemorrhages. The entire central portion of the abdominal cavity was occupied by a large mass reaching to the diaphragm in the left upper quadrant, coming into close relationship with the under surface of the liver and extending downward to the pelvic brim. The omentum was found riding over the mass, extending from the greater gastric curvature above to the transverse colon lying just below the most prominent portion of the tumor. The kidneys were found lying on either side of the neoplasm, somewhat distorted by its pressure, but not incorporated in it. No adrenal gland was found on the left side, that on the right side was in its usual position between the upper pole of the kidney and the under surface of the liver. The surface of the tumor was lobular and smooth. It was cystic to palpation in some areas and solid in others. It was gray with red splotches scattered here and there, and on section displayed areas of gray soft bloody tissue with other cystically dilated portions filled with bloody fluid. Other areas were semifluctuant, purplish and necrotic.

There was no gross evidence of metastatic involvement of the other abdominal, pelvic or thoracic viscera. The skeleton, however, showed widespread metastases. These were found in the sternum, ribs, femurs and skull and appeared as slightly elevated dark red patches of variable size and irregular shape. When the orbital plates were removed masses of soft dark red, friable tissue were found in the retro-orbital spaces. The amount of this foreign tissue was not sufficient to cause exophthalmos.

The gross anatomic diagnosis was neuroblastoma of the Hutchison type arising from the left adrenal medulla.

Histologic preparations revealed, in addition to the grossly visible metastases, involvement of the liver, kidneys and spleen. The tumor was seen to consist of masses of small round cells appearing not unlike lymphocytes. There were large areas of necrosis and hemorrhage. The cell type was uniform throughout, and the stroma was of variable quantity.

Examination under high power showed the cells to be neuroblasts, rather larger than lymphocytes, with relatively more cytoplasm and large vesicular deeply basophilic nuclei. They were arranged in clumps and strands. No typical "rosette" arrangement could be found.

This tumor conforms to the Hutchison type of neuroblastoma of the adrenal medulla.

GENERALIZED TUBERCULOSIS IN THE NEW-BORN HARRY M. STEEN

A woman, aged 27 years, was admitted to the obstetric ward of the Presbyterian Hospital in labor on Nov. 16, 1933, and delivered of a living full-term female child weighing 7 pounds and 9 ounces (3,430 Gm). The mother gave a history of a

severe respiratory infection occurring during the third month of pregnancy, at which time she was confined to bed for two weeks. The mother was seen for the first time in the prenatal clinic on July 17, she had the physical signs and roentgen evidence of a pleural effusion of the left side. The diagnosis on admission was questionable pulmonary tuberculosis, a diagnosis which was subsequently strengthened by roentgen studies and physical findings during her stay in the hospital. However, neither the roentgenologists nor the clinicians would commit themselves to a diagnosis of active tuberculosis. Two examinations of the sputum and one of the gastric residue disclosed no acid-fast bacilli.

The baby lived twenty-five days. The clinical course was as follows. On the second day, the baby had a temperature of 100.4 F. From the second to the fifth day, the fever ranged between 100.4 and 102.4. The baby was transferred from the obstetric ward to the pediatric service of Dr Charles A. Fife. On the fifth day, there was slight vaginal bleeding. The baby was thought to have hemorrhagic disease of the new-born, and 20 cc of the mother's blood was injected intramuscularly into the buttocks. The spleen was enlarged and extended out from the costal margin 1 cm. On the seventh day, the baby was placed at the mother's breast for the first time and received five feedings. On the eighth day, the vaginal bleeding stopped, and the temperature was 103 F. The baby was removed from the breast and placed on a formula. On the tenth day, the baby was slightly dehydrated, the spleen was larger, and the x-ray films showed a general fuzziness of the chest, especially about the shadows of the roots of the lungs. There was a decreased resonance of the apex of the right lung. On the eleventh day, the baby was more dehydrated, the spleen was larger, the liver was enlarged, and the abdomen was distended but soft. The Mantoux test was positive. On the eighteenth day, the febrile course continued, and there was a positive reaction to tuberculin (0.001 mg.). The liver was palpable almost to the level of the umbilicus. On the twenty-first day, the gastric residue and feces contained acid-fast organisms in large numbers. On the twenty-fourth day, resonance was impaired generally over the chest, and fine crepitant rales were heard all over the chest. Death occurred on the twenty-fifth day of life.

November 16, the Wassermann and Kahn tests of the cord were negative. November 20, the bleeding time was four minutes and thirty seconds, the coagulation time, three minutes. November 25, the hemoglobin was 110 per cent. The red blood cell count was 5,120,000, the white cell count, 11,650. The polymorphonuclears were 52, the small lymphocytes, 46, and the undifferentiated cells, 2 per cent. December 4, the hemoglobin was 106 per cent. The red blood cell count was 4,320,000, the white blood cell count, 12,150. The polymorphonuclears were 88, the small lymphocytes, 11, and the monocytes, 1 per cent. December 6, guinea-pigs inoculated with gastric residue showed tuberculosis. December 9, guinea-pigs inoculated with spinal fluid and blood cultures showed tuberculosis. Guinea-pigs given injections of the mother's milk disclosed no evidence of tuberculosis.

At necropsy general military tuberculosis was found. The brain and long bones were not involved.

Microscopically, the tubercles in the involved viscera appeared to be of the same age and were exudative. Acid-fast organisms were demonstrated in sections of the lungs, liver, spleen, kidneys and adrenals. Numerous sections through the umbilical vein showed no tuberculous involvement. The placenta was discarded at the time of delivery.

Recognizing that the five breast feedings and the injection of the mother's blood introduce a reasonable doubt, I still feel that the absence of clinical or roentgenologic evidence of open tuberculosis in the mother and the negative findings in guinea-pigs given injections of the mother's milk confirm the belief that this case is an example of transplacental infection.

PRIMARY (?) TUBERCULOUS PERICARDITIS A I RUBINSTEIN

A Russian Jew, aged 64, was admitted to Mount Sinai Hospital, Feb 6, 1933, complaining of precordial and epigastric pain. For about six years he had had hypertension, and attacks of precordial pain had often followed physical effort. These symptoms developed following right hemiplegia, from which recovery had been gradual but complete. He was fairly well nourished, dyspneic and moderately cyanotic at rest, the blood pressure was 202 systolic and 95 diastolic. The temperature was 99 F, the pulse rate was 104 and the respiratory rate 24. The supracardiac area was slightly broadened, the heart was moderately enlarged in all diameters, the sounds were rhythmic and of poor quality, with A2 markedly accentuated. There was evidence of diffuse fibrosis of the lungs, with râles at both bases, there were tenderness and rigidity of the right hypochondrium, and the liver was definitely palpable, there was slight pretibial edema.

The presumptive diagnosis was advanced arteriosclerosis, with coronary involvement, hypertension and decompensation, and early bronchopneumonia. Cytologic, chemical and serologic examination of the blood gave negative results. The urine was normal. Roentgen examination of the chest confirmed the physical evidence of moderate cardiac enlargement and also revealed congestion of the basal portions of the lungs. The electrocardiogram revealed evidence of myocardial involvement but not of acute coronary occlusion.

During the month's hospitalization, the temperature varied from 99 to 101 F. Venesection with various medicinal measures resulted in more comfort for the patient, with a drop in the systolic blood pressure to 160, but the anginal attacks became more frequent and severe and were accompanied by increased cyanosis and dyspnea. The pulmonary involvement steadily increased, accompanied by a gradual weakening of the cardiovascular mechanism. The patient died on March 6.

At autopsy the anterior surface of the pericardium was normal. The parietal pericardium was greatly thickened. About 100 cc of bloody fluid was removed from the pericardial sac, revealing a hemorrhagic pericarditis of the "bread and butter" variety involving the entire surface of the heart and ending abruptly at the point of reflection of the pericardium.

The mitral and aortic valves were normal except for moderate atheromatosis. The left ventricular wall was 1.8 cm thick. The right coronary vessels and the left circumflex branch showed some atheromatous change but no encroachment on their lumens. In addition, the left descending branch in one region showed definite constriction of the lumen. Histologically, the epicardial layer was thickened by multiple tubercles of the productive variety. The tuberculous process stopped abruptly at the myocardial border except in a few areas in which a slight infiltration of round cells in the outer muscle layers was noted. There was extensive fibrosis of the lungs with diffuse pneumonitis, terminal infarction and atelectasis. Careful search for other tuberculous foci revealed only a few prominent fibrotic mediastinal lymph nodes some distance from the pericardium. Histologic study revealed a questionable reactivation of very old tuberculous lymphadenitis.

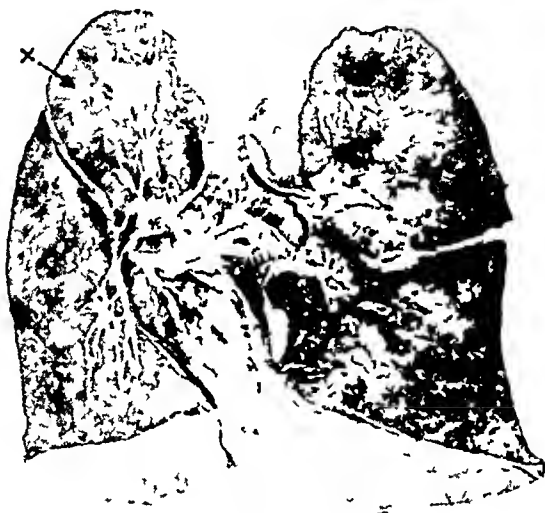
CLINICAL AND PATHOLOGIC NOTES ON A CASE OF HIALID PULMONARY TUBERCULOSIS F MAURICE MCPHEDRAN and ESMOND R LONG

Perfectly healed tuberculosis of the adult type and of unknown clinical course is frequently seen at necropsy. Tuberculosis of known course is seldom seen. It seems worth while, therefore, to put a case, recently observed, on record.

A colored man, 34 years old, entered the clinic of the Henry Phipps Institute in August 1928 with a history of cough of five months' duration. He was well nourished, weighing 187 pounds (84.8 Kg). He had lost 18 pounds (8.2 Kg) some months previously, but shortly regained the loss. Abnormal physical signs in the chest were inconstant. Roentgen examination showed groups of very light spots in the posterior fourth and fifth interspaces on both sides. Tubercle bacilli in small numbers were found in the sputum in two of three examinations in August.

In 1929, 1930 and 1932 he had acute respiratory symptoms, with roentgen evidence of bilateral basal nontuberculous bronchopneumonia and associated basal rales. The groups of light spots visible in the upper lobes in 1928 were no longer apparent in the x-ray films, nor were physical signs referable to this part of the chest. Repeated examinations of the sputum between August 1928 and June 1934 disclosed no tubercle bacilli. During these years headaches, polyuria and visual defects developed. Roentgen examination of the skull disclosed an enlargement of the sella turcica. An operation for tumor of the hypophysis performed on June 28, 1934, was unsuccessful and the patient died in a state of shock a few hours afterward.

In addition to the hypophyseal tumor—an acidophilic cell adenoma—the autopsy disclosed significant changes only in the lungs. In the posterior parts were edema and early inflammatory change. The lungs were fixed by filling them through the trachea with Kaiserling's solution 1, and after thorough hardening they were cut into slices 1 cm thick. In the outer portion of the middle third of the right upper lobe was a scar which had an anthracotic margin with radiating extensions out-



Isolated healed tuberculous lesion of adult type in the upper lobe of the left lung, believed to have been the source of the tubercle bacilli in the sputum five years previous to the death of the patient (death from tumor of the brain)

ward. The scar measured 7 by 9 by about 7 mm. It surrounded a softer, white, partly fibrous mass, measuring 6 by 3 by 4 mm. No changes, fibrous or otherwise, were found in the apex, but there was a calcified tubercle, measuring 2 by 3 by 2 mm, just internal to the pleura directly posterior to the scar described.

No other significant gross changes were found in this lobe. In the right lower lobe was a calcified tubercle measuring 8 by 4 by 8 mm. In the regional pulmonary lymph nodes was a collection of hard calcified masses, totaling 1 cm in diameter, and a calcified mass 8 mm in diameter was present in a lymph node below the right main bronchus close to the bifurcation. Congestion, edema, thrombotic emboli, early hemorrhagic infarcts and early inflammatory consolidation were seen elsewhere in the lungs.

The diagnosis was healed tuberculosis of the childhood type in the right lower lobe and healed ulcerative tuberculosis of the adult type in the left upper lobe. Microscopic sections of the latter lobe disclosed a dense fibrous wall and a necrotic center, infiltrated at the margins with lymphocytes and fibroblasts. The usual

histologic criteria of activity were absent. Tubercle bacilli could not be found (Obviously, because of the fixation necessary in finding the lesion, experiments in inoculation were ruled out). The bronchus leading to the region of the scar was somewhat contracted by fibrous tissue, and its lumen was almost filled by accumulated, in part degenerated, epithelial cells. The artery to the part was much thickened, with a greatly reduced lumen. In the regional lymph nodes, in addition to anthracosis, there was slight fibrous scarring.

It was believed that the scar in the right upper lobe represented the ulcerative lesion responsible for the sputum containing tubercle bacilli six years before. It is altogether probable that this lesion in turn was secondary to the subpleural lesion described, and that the latter was the first lesion of reinfection.

CHANGES IN THE PITUITARY FOLLOWING THYROIDECTOMY IN RATS. ISOLD T. ZICKLER, LEWIS W. DAVISON, THOMAS B. KILMER and CLARENCE S. LIVINGOOD II

A paper on this subject was read before the American Society for Experimental Pathology, the abstract of which appears on page 159.

DYSONTOGENETIC ORIGIN OF BASAL CELL CARCINOMA. JOSEPH MCFARLAND, E. F. CICCONI and JOSEPH GIEHRTLER

We endeavored to confirm or refute Glasunow's theory that the basal cell carcinoma develops from imperfections in the closure of the embryonal facial fissures. A consideration of the theories as to the nature and origin of the basal cell tumors and of the diversified histologic structure suggests that the tumors arise from something more primitive than the perfected epiderm. Attention is also called to the fact that no basal cell tumor has ever been produced experimentally.

Glasunow collected 254 cases of basal cell carcinoma, 112 of which after histologic confirmation had their exact localization on a diagram of the face. Their distribution conformed almost perfectly with the location of the embryonal facial fissures.

We culled 50 cases from the material in the Philadelphia General Hospital and 65 cases from that in the American Oncologic Hospital and similarly indicated the localizations on a facial diagram. With the exception of a few scattered cases the carcinomas tended to fall into positions in fair correspondence with those of the embryonal facial fissures.

As there is lack of agreement as to the histology of basal cell tumors, Ewing's criterion was adopted, viz. "The normal transformation into squamous cells entirely fails."

In order to support the evidence by comparison, two other types of dysontogenetic lesions, similarly plotted, were studied. These were the so-called sequestration dermoids and the facial mixed tumors. For the former, Forgue's diagram was borrowed. With the exception of one or two lesions aberrant on the forehead, the correspondence was complete. The aberrant lesions may have been sebaceous cysts mistaken for dermoids.

One of us (Dr. McFarland) reviewed the entire literature of mixed tumors of the face, lip, nose and supra-orbital regions, noting their reported localizations, and plotted them on a similar diagram. Again, with very few exceptions, which may relate to errors in diagnosis, the tumors conform to the distribution of the embryonal facial fissures.

So the distributions of the basal cell tumors, the sequestration dermoid cysts and the mixed tumors are the same, and the number of aberrant cysts and tumors is not of sufficient importance to discredit the theory.

Another comparison to act as a control was made by similarly plotting 100 cases of squamous cell lesions of the face (50 from each institution). The result is extraordinarily different, and the distributions have no relation to the fissures.

Conclusion—The results obtained are in complete accord with those of Glasunow and support his theory that basal cell carcinoma is of dysontogenetic origin and arises from ectodermal cells sequestered in the concrescence of the embryonal facial fissures.

Book Reviews

On the Occurrence of Lymphogranulomatosis (Sternberg) in Sweden 1915-1931 and Some Considerations as to Its Relation to Tuberculosis
By Martin Uddstromer Acta Tuberculosea Scandinavica, Supplementum 1
Copenhagen Levin & Munksgaard, 1934

Cases of lymphogranulomatosis in Sweden from 1915 to 1931 numbered five hundred and forty-eight. The average rate of incidence is two hundred and eighty-eight times less than that of tuberculosis for the years from 1915 to 1930. The absolute geographic distribution of lymphogranulomatosis coincides with the more densely populated areas. Regional differences are found, but are evenly distributed between the rural and urban populations. Several facts argue against the contagiousness of lymphogranulomatosis, social and hygienic conditions do not seem to be of importance.

Males preponderate over females in number, and this is accentuated in childhood, speaking against occupational factors in the relative sexual morbidity. Ovarian hormones as protective influences for the female do not seem to play a part.

Age is held to be a predisposing factor, the disease being exceedingly infrequent during the first two years of life, not common up to the age of puberty and showing a marked increase immediately thereafter. The peak of the curve for age does not form a maximum, except for females. The disease occurs frequently among all age groups, in males with increased incidence to form a maximum at about 60 years. The relative age at incidence in lymphogranulomatosis conforms only in part with that in tuberculosis.

As to duration, 60 per cent of the patients died in less than two years, and 87 per cent in less than four years. The data for those aged 60 years or more show a shorter duration.

"Neither in geographical distribution nor in family histories, clinical and post-mortem cases do we find a coincidence of LG with tuberculosis that can lend support to any connection between the two diseases beyond the fact that LG favors the setting in of military tuberculosis, where in all post-mortem cases older tuberculous changes have been present." Tuberculin reactions are more frequently negative than is generally stated.

A percutaneous portal of entry is not favored, but the material seems to lend support to a possible gastro-intestinal portal in some cases, this tract being involved in at least one third of the cases. Splenomegaly is found in two thirds of the cases. The peritoneum is rarely involved. The abdominal lymph nodes, especially the retroperitoneal nodes are preponderantly affected. The lungs are affected in about one third of the cases, and usually the nodular form of the disease is encountered. It is difficult or even impossible at times to differentiate either clinically or at postmortem examination between lymphogranulomatosis and tuberculosis. The hemorrhagic diathesis is not often encountered. Leukocytosis occurs in many cases, being somewhat accentuated in advanced stages. Neutrophils are likewise increased, and the great majority of patients show normal or decreased eosinophils. Anemia is generally mild, but is present in about one half of the advanced cases and in about one third of the early cases. The sedimentation reaction always shows raised values, which are often marked and may be considered of prognostic importance. Normal values must not be taken as a sure sign of prolonged duration. High rates occur more frequently in lymphogranulomatosis than in tuberculosis.

Records of several rare cases seen clinically and post mortem are given. An extensive bibliography is included.

The reviewer wishes to call attention to this monograph as being the most detailed and exhaustive survey of a single phase of lymphogranulomatosis yet published. The most striking observation resulting from this work would appear to be that there is no coincidence between lymphogranulomatosis and tuberculosis other than the occurrence of terminal miliary tuberculosis in association with some cases of lymphogranulomatosis.

Experimental Bacteriology in Its Applications to the Diagnosis, Epidemiology, and Immunology of Infectious Diseases Volumes I and II By Dr W Kille, Director of the Institute for Experimental Therapy and of the Chemicotherapeutical Research Institute "Georg Speyer-Haus," Hon. Professor at the University of Frankfurt, and Dr H Hetsch, Professor at the Institute for Experimental Therapy, Frankfurt. Translated from the seventh, completely revised German edition by Dagny Erikson, the English version, incorporating further revision, edited by John Eyre FRS (Edin), FZS, MD, MS, DPH, Director of the Bacteriological Department, Guy's Hospital, Professor of Bacteriology, University of London. Price, \$16. Pp. volume I, 592, volume II 613, with 118 plates and 200 text figures. New York. The Macmillan Company, 1935.

The first edition of this book appeared in 1906. Through the years the successive editions have been accepted as giving a true and authoritative presentation of the science and art of bacteriology in theory and practice according to the traditions of the Koch school. Translations have been made into several European languages, the most recent being this into English from the seventh and last German edition. The first volume begins with general bacteriology and immunology, after which come twenty-seven chapters on infectious diseases, nearly all of bacterial origin. The second volume deals with tuberculosis, leprosy, spirochetal and protozoan diseases, diseases due to filtrable viruses, infectious diseases of unknown causes (in which are included beriberi, pellagra and scurvy) and diseases due to fungi and yeasts. Because the two volumes constitute an organic whole it is unfortunate that each is treated separately in the matter of pagination and index, especially as there are no indications in the titles as to what each volume contains. There should have been consecutive pagination and one common index. The translation impresses one favorably. The illustrations are admirable, particularly the colored plates. The old names of bacteria are used consistently and exclusively, the indexes do not contain any newer names, such as *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Eberthella*, *Escherichia* and *Clostridium*. Bacterial dissociation or pleomorphism is not discussed, the old monomorphic conception holds full sway. In several other respects also the book has failed to keep step with the advances, e. g., in its discussion of bacterial food poisoning, of typhus fever, of brucella infection, of streptococci in relation to epidemic sore throat and to specific toxin production in erysipelas. The main value of the book to the student of microbiology and infectious diseases lies in its portrayal of the wonderful developments in those fields in Germany under the influence of the great pioneers of the Koch era.

The Kidney in Health and Disease In Contributions by Eminent Authorities Edited by Hilding Berglund, MD, Stockholm, Sweden, formerly Chief of the Department of Medicine at the University of Minnesota, and Grace Medes, PhD, Research Biochemist in the Lankenau Hospital Research Institute, Philadelphia, with the Collaboration of G. Carl Huber, MD, Warfield T. Longcope, MD, and Alfred N. Richards, PhD, MD. Cloth. Price, \$10. Pp. 754, with 163 engravings. Philadelphia. Lea & Febiger, 1935.

This book contains the revised and amplified papers contributed to the symposium on the kidney at the University of Minnesota in the summer of 1930. This symposium was organized by Hilding Berglund, then professor of medicine in the university. Since his return to Sweden the editorial work has been completed by Grace Medes and her collaborators. The book contains forty-four articles, and

6

there are forty-one contributors. The contents are divided into six parts: the anatomy and physiology of the kidney, the clinical aspects of renal functions, Bright's disease and various other pathologic renal conditions, albuminuria and edema, ocular changes in Bright's disease and the clinical aspects of Bright's disease. To attempt to review the individual articles is out of the question. The authors represent well the active investigators and students of the kidney in this country, European visitors contribute several articles. The editorial work has been done carefully. There are good indexes. The book is an example of a high standard of book-making. It gives a comprehensive review of present knowledge of the kidney in health and disease which will prove helpful to all who are interested in that field.

Books Received

A BIBLIOGRAPHY OF THE POINTE-À-PÂTE SINE MORBUS GALLICUS BY GIROLAMO FRACASTRO OF VIGONA Leona Baumgartner and John F. Fulton Price, \$5 Pp 157, with 10 illustrations New Haven, Conn Yale University Press, 1935

THE INTERNATIONAL CANCER RESEARCH FOUNDATION, REPORT OF ACTIVITIES DURING 1934 Pp 79

MEMORIAL MEETING IN HONOR OF WILLIAM HENRY WELCH, HELD AT THE UNIVERSITY CLUB, TUESDAY, MAY 22, 1934 Pp 44, with 1 illustration Baltimore The University Club, 1935

KEY-CATALOGUE OF PARASITES REPORTED FOR CARNIVORA (CATS, DOGS, BEARS, ETC) WITH THEIR POSSIBLE PUBLIC HEALTH IMPORTANCE C W Stiles and Clara Edith Baker National Institute Health Bulletin No 163 (Continuation of Hygiene Laboratory Bulletin Series), December 1934 Price, 20 cents Pp 913-1223 (pagination continuous with that of Bulletin 159) For sale by Superintendent of Documents, Washington, D C United States Government Printing Office, 1935

COLLECTED REPRINTS FROM THE LABORATORIES OF THE MOUNT SINAI HOSPITAL, NEW YORK, LOUIS GROSS, M D, DIRECTOR, 1934

REPORT OF THE LABORATORY AND MUSEUM OF COMPARATIVE PATHOLOGY OF THE ZOOLOGICAL SOCIETY OF PHILADELPHIA IN CONJUNCTION WITH THE SIXTY-THIRD ANNUAL REPORT OF THE SOCIETY Herbert Fox, M D, Pathologist Pp 32 1935

EXPERIMENTAL STUDIES ON CANCER Carl Voegtlin and Others National Institute of Health Bulletin No 164, January 1935 Price, 10 cents Pp 58, with 4 plates For sale by Superintendent of Documents, Washington, D C Washington, D C United States Government Printing Office, 1935

CONTRIBUTION A L'ETUDE DE LA VARIABILITE DU VIRUS TUBERCULEUX P Denys Pp 90, with 6 figures Louvain Imprimerie Saint-Alphonse, 1935

TWO RARE INSTANCES OF CARDIOVALVULAR DISEASE PRESUMPTIVELY SYPHILITIC IN ORIGIN

ARTHUR R SOHVAL, M D *

NEW YORK

In a report of two cases of cardiac gummas,¹ acquired tertiary syphilis of the heart² is considered synonymous with gummatous myocarditis, localized or diffuse. This is in conformance with current concepts. The characteristics of the lesions are definite, and the pathologic diagnosis is generally a simple one.

Two additional cases were recently encountered which lack the accepted diagnostic criteria of gumma, although the collected evidence points to a presumptive diagnosis of tertiary syphilitic heart disease.

This raises a question as to whether the gummatous lesion represents the sine qua non of cardiac syphilis. Enlightenment on this issue may be obtained by analogy with aortic syphilis. Conner,³ in a masterful survey of the development of knowledge of cardiovascular syphilis, described the bitter and finally successful "struggle for the recognition of the specific nature of syphilitic aortitis." Yet, even today the diagnosis of syphilitic aortitis is at best presumptive and not absolute, since the lesion is usually not gummatous and the specific organism has only rarely been recognized in it.

The two cases now reported present unusual lesions of the mitral valve. As far as can be determined, lesions of the same type have not been described hitherto. Their nature together with certain points of similarity in the findings in proved cases of syphilitic heart disease reported in the literature suggests a clue as to their etiology.

REPORT OF CASE 1

A 51 year old Austrian was admitted to the medical service of Dr. George Baehr on May 1, 1931, complaining that he had had a nocturnal cough productive of greenish sputum for four weeks. Five days previous to admission he was seized with an agonizing and suffocating sense of constriction in the chest. From

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This work was aided by a grant from the Lucius N. Littauer Foundation and by one from the Emanuel Libman Fellowship Fund in memory of Adele Schiff

1 Sohval, A. R. Arch. Path., to be published

2 Exclusive of aortitis with commissural involvement and omitting Virchow's type of diffuse myofibrosis (nondiagnostic)

3 Conner, L. A. J. A. M. A. 102:575, 1934

then on there were moderate difficulty in breathing, "tightness" in the chest and occasional vomiting

He had pneumonia in childhood, and at the age of 20 years he suffered from gonorrhea and arthritis

The patient was acutely ill. The temperature was 101 F, the pulse and respiratory rates were, respectively, 44 and 28 per minute. The left pupil was larger than the right, both were round both reacted sluggishly to light and were fairly active in accommodation. Crackling rales were auscultated throughout the lungs and were most numerous at the bases posteriorly. By percussion, the heart was found considerably enlarged to the left, the apex being in the sixth left intercostal space in the anterior axillary line. A diastolic murmur was present along the left border of the sternum in the third and fourth intercostal spaces. The rhythm was regular except for many interpolated beats consisting of only one sound. Pulsations corresponding to these could be seen over the region of the internal jugular veins but could not be palpated over the radial artery at the wrist. The pulsations in the latter were of Corrigan's type and equal. By percussion the liver was found to extend 1 fingerbreadth below the free border of the ribs. An old penile scar was present. On the left calf was a superficial flat scar about 3 cm in diameter with a pigmented border. Neurologic examination disclosed nothing except a positive Hoffmann sign on the left, absence of the plantar and cremasteric responses and pupillary changes as noted.

The Wassermann and Kahn reactions of the blood were negative. The spinal fluid was normal cytologically and serologically (the Wassermann reaction and colloidal gold test were negative). The systolic blood pressure varied between 156 and 190 mm of mercury, and the diastolic, between 68 and 84. Hemoglobin was 76 per cent. There were 10,000 leukocytes per cubic millimeter of blood, of which 90 per cent were polymorphonuclear neutrophils. The blood contained 27 mg of urea nitrogen per hundred cubic centimeters. The urine contained a moderate amount of albumin and occasional leukocytes.

An electrocardiogram showed complete auriculoventricular dissociation. The auricular rate was about 110 per minute and the ventricular rate about 58. There was deep inversion of the T waves in all leads. Myocardial damage was suggested by the tracings.

On the third day after admission the patient suddenly lapsed into stupor and died on the following day.

The final diagnosis was syphilitic aortitis, aortic insufficiency and complete heart block.

Autopsy—The cardiac findings are given in detail. The heart (fig 1A) was considerably enlarged and weighed 500 Gm. There was no evidence of pericarditis. The right auricle and ventricle were slightly dilated. The tricuspid and pulmonary cusps were normal. The root of the pulmonary artery was normal.

The left auricle was enlarged and somewhat hypertrophied. Its endocardium was thickened and whitened. The posterior mitral leaflet was normal. The anterior mitral leaflet was whitened, and its upper two thirds definitely thickened. This portion felt cartilaginous. When this leaflet was split open it was found to measure 4 mm at the auricular attachment. The cartilaginous structure was seen to continue upward behind the auricular wedge to become continuous with the root of the aorta, on the one hand, and with the septum fibrosum, on the other. The left ventricle was somewhat hypertrophied and considerably dilated. The posterior papillary muscle appeared to be atrophic and showed hemorrhages at its apex. The anterior papillary muscle was of normal size but also contained hemorrhages at its apex. The outflow tract was decidedly elongated.

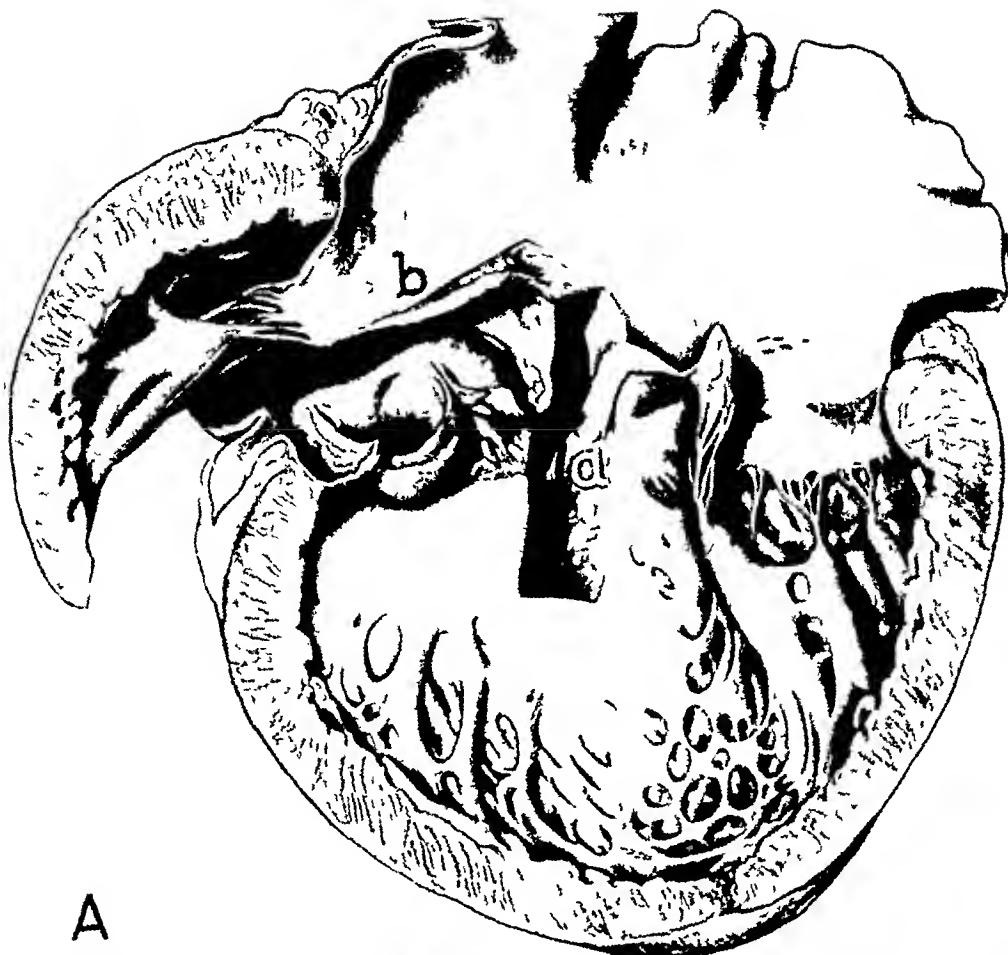


Fig 1 (case 1) —*A*, view of the left side of the heart showing extension of the dense cartilaginous-appearing lesion into the contiguous membranous septum (*a*) and anterior mitral leaflet (*b*) *B*, fibrosa of the anterior mitral flap under high power magnification, showing perivascular foci of lymphocytes, plasma cells and large mononuclear cells in a dense hyaline stroma *C*, the same structure on the auricular aspect under high power magnification, showing hyperplasia of the elastica (*a*), numerous vessels (*b*) and diffuse infiltrations with round cells. The fibrosa layer is at *c*. Both *A* and *B* show MacCallum's stain for elastica and Van Gieson's stain. Magnification, 16 mm, $\times 200$

The aortic cusps were of usual size. The right cusp showed a rounded thickening occupying approximately 1 cm of the middle portion of its free edge. The right posterior commissure was somewhat fused. The posterior leaflet showed a rounding and shortening. The posterior extremity of the left leaflet showed a similar lesion, and the left posterior commissure was the site of a moderate separation (2 mm). The endocardium over the undefended space was thrown up into rugosities. These prominences felt somewhat rubbery, and the undefended space was opaque to light. The rugosities over the endocardium of this space were continuous with a similar lesion on the ventricular surface of the anterior mitral leaflet and in the region of the left posterior commissure. The lesion continued through the separation of this commissure and could be seen very markedly within the pockets of the component cusps. The sinus pocket of the right cusp also showed striations and rugosities which, however, were less marked. The right-left commissure showed a separation approximately 1 mm in width.

The aorta measured 8 cm at its root and showed mild sclerotic plaques. Coronary arteries and their branches showed discrete mild atheromatous changes without narrowing.

*Microscopic Examination*⁴—Aorta. The ascending portion above the root presented only mild alterations. A slight fibro-elastic proliferation was present in the intima. There were many capillaries surrounded by small areas of fibrous tissue in the outer half of the media. The vasa vasorum of the adventitia were thickened by a hyperplastic intimal process with some narrowing of the lumens and perivascular accumulations of plasma cells and lymphocytes.

Mitral and Aortic Valve. The sinus wall of the posterior aortic cusp, containing the root of the aorta and the annulus fibrosus of the valve, was liberally involved by foci of lymphocytes, fibroblasts, large mononuclear and plasma cells and capillaries, which continued down to implicate the ring and base of the valve cusp and the dense fibrous tissue behind the annulus. From here this inflammatory process, set in whorls of connective tissue, extended through the intervalvular fibrous layer into the fibrous layer of the anterior leaflet of the mitral valve. The latter structure was therefore markedly broadened. The aortic cusp was normal (except for the inflammatory lesion at the base). Its tip was expanded into a non-inflammatory, avascular, fibro-elastic, bulbous formation. Superimposed on the sinus wall was a well developed fibrous proliferation continuous with that of the intima of the aorta just described.

Anterior Cusp of Mitral Valve. The leaflet was markedly and diffusely widened for the proximal two thirds of its length by a dense hyaline connective tissue expansion of the fibrous layer continuous with that described in the intervalvular fibrosa. It was arranged in whorls and was extensively involved by numerous small capillaries (some with well developed elastic coats) and perivascular aggregations of plasma cells, lymphocytes and larger mononuclear cells (fig 1 B). At each border of the fibrosa there was a condensation of the inflammatory process which formed the base of a band of fibrous tissue proliferation, fairly well supplied with elastica, vascularized, infiltrated, and more prominent on the auricular side, where the vessels were larger and had thicker walls (fig 1 C). The original auricular and ventricular elastic lamellae tended to be frayed and reduplicated. This chronic inflammatory process likewise infiltrated the tip of the left auricular myocardial wedge. The Levaditi stain was negative.

4 Blocks of cardiovalvular tissue in this and the following case were cut according to the standardized procedure of Gross, Antopol and Sacks (Arch Path 10 840, 130). Sections were stained with hematoxylin and eosin and in duplicate with Weigert's elastic and Van Gieson's connective tissue stain.

Posterior Cusp of Mitral Valve The ring and valve were normal

Tricuspid Valve and Interventricular Septum The ring and proximal portion of the leaflet contained a generous number of capillaries and round cells. The fibrous septum was markedly thickened by a sclerocellulovascular process identical with that described in the anterior mitral flap. The region of the bundle of His was the site of a more focal and extensive, though similar, involvement with elastic scar tissue and obliterative arteriolar lesions. There were large numbers of fibroblasts. In its midst were seen remnants of degenerated muscle fibers.

Pulmonary Valve The ring, valve and artery were normal

Posterior Papillary Muscles There were extensive areas of deeply staining myocardial fibers of hyaline appearance with loss of nuclei and striations, indicative of massive necrosis. In general, their form was preserved, although in occasional foci they were completely disintegrated. Between the muscle bundles were extravasated erythrocytes and marked infiltrations of leukocytes, almost entirely polymorphonuclear, and arranged in more or less linear distribution. There was a moderate amount of perivascular fibrosis.

Left Auricle The auricle was normal except for some sclerotic thickening of the endocardium.

Other significant findings at autopsy included congestion and edema of the lungs and congestion of the liver and spleen. Evidence of extracardiac syphilis was not discovered.⁵

COMMENT ON CASE 1

Because of the history of gonorrhea, the cardiac findings, Corrigan pulse, penile scar, pupillary signs and reflex changes, this patient was regarded clinically as presenting syphilitic aortitis with aortic insufficiency. Necropsy not only confirmed this impression but disclosed a lesion of cartilaginous hardness in the mitral valve and septum fibrosum with destruction of the bundle of His. While a lesion in the latter situation might have been surmised from the clinical and electrocardiographic evidence of heart block, the alteration in the mitral valve was a completely unexpected finding since it gave rise to no clinical signs.

Until recently, the existence of proof of syphilis of the mitral valve was denied.⁶ To date there are but two authentic cases on record, Friedman⁷ and Staemmler⁸ each reported one case of mitral syphilis in combination with syphilitic aortitis and aortic insufficiency. The Wassermann reaction of the blood was positive in both. In each case the lesion consisted of a firm thickening of the anterior leaflet by dense inflammatory connective tissue. There were diffuse infiltrations of round cells. Military areas of necrosis and young capillaries were present. Giant cells were found only in Staemmler's case. Schmorl⁹

5 The brain was not examined

6 Breitmann M. *Gaz d hop* **76** 213, 1903. Steinberg, M. J. *Am J Syph* **12** 316, 1928.

7 Friedman, W. *Proc New York Path Soc* **24** 24, 1924.

8 Staemmler. *Verhandl d deutsch path Gesellsch* **25** 262, 1930.

9 Schmorl, in discussion of report by Staemmler.⁸

cited Geipel's case of lesions in the mitral valve identical with those in Staemmler's case. Other cases of so-called syphilitic mitral valve disease are most probably instances of associated endocarditis.

In view of the extreme rarity of syphilitic mitral valvulitis, great caution must be exercised in accepting a new case, especially in the absence of coagulation necrosis. However, certain features point strongly to syphilis as the etiologic factor in the instance under discussion.

The aortitis and aortic insufficiency were typically syphilitic. The negative serologic data do not vitiate the diagnosis since the serologic findings in a fair percentage of cases (about 20 per cent) of proved cardiovascular syphilis are negative.

Blocks of cardiovalvular tissue were cut according to the standardized method of Gross, Antopol and Sacks.¹⁰ These included one section taken through the root of the aorta, the posterior aortic cusp and the anterior flap of the mitral valve. From a study of the normal topography in this region (fig. 2) it is not difficult to see how the medial and adventitial lesions of syphilitic aortitis may descend to involve the annulus fibrosus of the aortic valve, the loose tissue behind it, the intervalvular fibrous layer and the fibrosa of the anterior curtain of the mitral valve.

Microscopic examination of this crucial section enabled one to trace the entire process as it extended from the root of the aorta into and behind the aortic annulus, through and alongside the intervalvular fibrosa and into the fibrosa of the aortic flap of the mitral valve. These observations confirm the mode of extension noted by Staemmler⁸ in his case. They likewise represent a much later stage than the early mild process noted subsequently in case 2 (fig. 4 *A* and *B*).

The cause for the complete heart block noted during life was found in the densely fibrous and granulomatous lesion in the membranous portion of the interventricular septum involving and destroying the bundle of His. This infiltration had the same gross and histologic characteristics as those noted in the anterior flap of the mitral valve and root of the aorta with which it was continuous. It likewise most probably originated by extension from the root of the aorta.

To the twelve instances of heart block due to cardiac syphilis collected by Major¹¹ are added those of Handwerch,¹² Holterdorf,¹³ de Marval and Vivoli,¹⁴ Cleland¹⁵ and Kux,¹⁶ bringing the total number

10 Gross, L., Antopol, W., and Sacks, B. *Arch. Path.* **10**: 840, 1930.

11 Major, R. H. *Arch. Int. Med.* **31**: 857, 1923.

12 Handwerch, C. *München med. Wchnschr.* **56**: 916, 1909.

13 Holterdorf, A. *München med. Wchnschr.* **63**: 1651, 1916.

14 de Marval, L., and Vivoli, D. *Rev. Soc. argent. de biol.* **2**: 425, 1926, *Rev. Soc. de med. int. y Soc. de fisiol.* **2**: 397, 1926.

15 Cleland, J. B. *M. J. Australia* **1**: 540, 1927.

16 Kux, E. *Ztschr. f. Kreislaufforsch.* **24**: 1, 1932.

of recorded cases to seventeen. While typical isolated cardiac gummas involved the conduction system in the vast majority of these cases the lesion in the instance reported by Vaquez and Esmem¹⁷ closely resembled that found in this case in the predominance of sclerotic and absence of necrotic processes. They accordingly designated the lesion as a sclerogummatous one despite the fact that giant cells and coagulation necroses were absent.

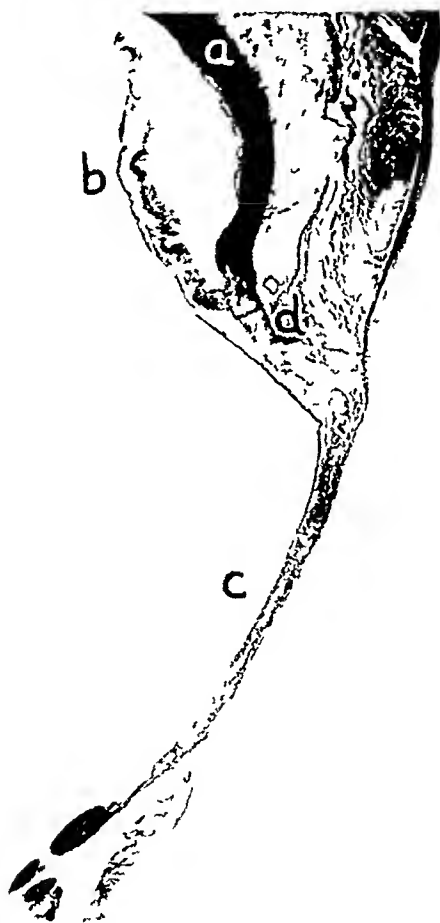


Fig. 2—Reproduction from Gross and Kugel (*Am J Path* 7 445, 1931, fig 6), showing the topography of the normal aorta (a), posterior aortic valve (b) and anterior mitral valve (c). This indicates the path of extension of syphilis from the root of the aorta through and behind the intervalvular fibrosa (d) to the aortic leaflet of the mitral valve. Weigert's stain for elastica and Van Gieson's connective tissue stain were used. Magnification, 75 mm, $\times 4$.

In the light of the foregoing observations, it is evident that the lesion in the anterior leaflet of the mitral valve in this case originated from a syphilitic process at the root of the aorta and was continuous with an identical lesion in the septum fibrosum. It consisted essentially

17 Vaquez and Esmem. *Presse med* 15 57, 1907

of a well scarred granuloma, presumably syphilitic. The absence of positive Levaditi stains does not render this less likely, since such is the rule even in authentic cases. The close resemblance of the valvular lesion to that noted by Friedman⁷ and Stammlei⁸ (differing essentially in the absence of coagulation necrosis and the predominance of fibrosis) is very suggestive. The striking similarity of the lesion in the conduction system to that observed by Vaquez and Esmein¹⁷ further strengthens the probability that the condition was syphilitic.

The advanced sclerotic condition of the lesion lacking coagulation necrosis and giant cells may indeed signify that a previously gummatous infiltration is now undergoing healing and scarring. On the other hand this type of lesion may simply represent syphilitic invasion of the fibrous structures of the heart (valve and fibrous septum) from a contiguous process at the root of the aorta.

REPORT OF CASE 2

A 20 year old white woman, unmarried, was admitted to the hospital under the care of Drs. H. Abramson and B. S. Oppenheimer on March 29, 1930, complaining that she had had "bronchitis," dyspnea, hemoptysis and pretibial edema for three weeks. Two years prior to admission she was found to have a "leaking" heart. Serologic examination of the blood and spinal fluid had been performed and the results reported as negative. She had a paratyphoid infection at the age of 4 and influenza during the pandemic.

She was admitted to the hospital in an almost comatose condition. The temperature was 98.6 F, the pulse rate was 128 and the respiratory rate 18 per minute. She was dyspneic and pale. Marked pulsation of the peripheral arteries of the extremities and neck was apparent. The left internal jugular vein was prominent. There was edema of the legs, thighs, sacrum, back, left hand and left breast. The pupils were dilated and did not respond to light. There were physical signs of an effusion in the left pleural cavity. Rales were heard diffusely throughout both lungs. The heart was enlarged, its action was regular. Systolic and diastolic murmurs were heard over the aortic area. The liver was tender and palpable as far as the umbilicus. She continued to grow worse and died several hours after admission to the hospital.

The systolic blood pressure had been 100 and the diastolic 0. Hemoglobin was 60 per cent, with 2,560,000 erythrocytes and 30,000 leukocytes per cubic millimeter of blood. The differential count was 77 per cent polymorphonuclear cells and 23 per cent lymphocytes.

The final diagnosis was myocardial decompensation, chronic cardiovalvular disease and aortic insufficiency.

Autopsy—The pericardial cavity contained approximately 100 cc of clear amber-colored fluid. The heart (fig. 3) weighed 500 Gm. It was much elongated and presented a marked dilatation of both ventricular cavities. The right auricle was moderately dilated. The tricuspid valve, pulmonary cusps and pulmonary artery were normal.

The left auricle was slightly dilated, its endocardium was markedly whitened. The aortic leaflet of the mitral valve was firm and thickened. The chordae tendineae had normal insertions into the free margin of the cusp. The posterior papillary muscle showed marked thickening of its endocardium and had a dense

whitish appearance. The left ventricle was moderately hypertrophied. The apex of the left ventricle, near the septum, contained an adherent firm thrombus. The outflow passage had a dense white endocardium, and its myocardium was scarred.

The ventricular surface of the aortic leaflet of the mitral valve was the seat of a diffuse deposit of firm consistency, pearly color and finely striated surface. This deposit extended up the outflow passage over the membranous septum to the base of the right and posterior aortic cusps, where it extended for a very short distance on these cusps. There was a striking defect of the right cusp in the form of a complete absence of the entire half adjacent to the posterior cusp. Only the line of insertion of this part of the cusp remained. It was situated 1 cm below the original annulus fibrosus, which was still visible. The posterior cusp, aside from the moderate thickening and a small deposit of material similar to that

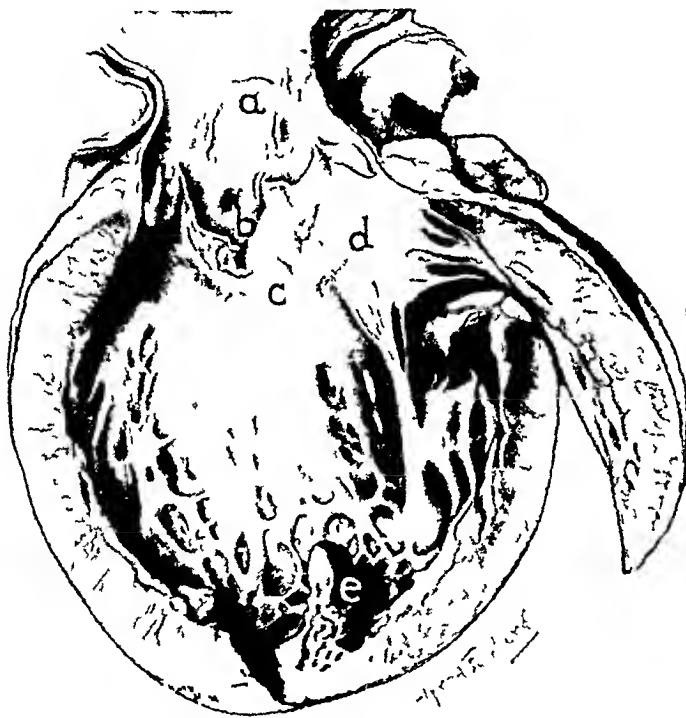


Fig 3 (case 2) —View of the left side of the heart showing syphilitic aortitis (a), loss of substance of the posterior portion of the right aortic cusp (b) and extension on to the endocardium over the membranous septum (c) and the aortic leaflet of the mitral valve (d). An adherent mural thrombus is shown at the apex (e).

already described, presented nothing remarkable. This was also true of the left cusp. The aortic valve commissures appeared normal except in the region of the defect, where there appeared to be some slight separation.

Rising from the region of the posterior right commissure there was a more or less circular, closely ridged elevation about 2.5 cm in diameter, which appeared to be continuous through the absent portion of the cusp with the striated deposit on the membranous septum and the anterior mitral leaflet. The remainder of the aorta showed practically no change. The coronary orifices were normal. The left anterior descending and left circumflex coronary arteries were patent throughout although moderately sclerotic.

Microscopic Examination—Aorta There was marked fibro-elastic proliferation of the intima. The architecture of the media was tremendously distorted. There were innumerable irregular scars, chiefly perivascular in location. The middle coat was extensively capillarized, and occasionally a thick-walled arteriole was observed with marked obliterative hyperplasia of the intima. The elastic fibers were distorted, fragmented, twisted and in many areas completely absent. Muscle fibers had largely disappeared. There was a sparse number of lymphocytes and mature fibroblasts, chiefly in the perivascular regions.

The adventitia was markedly thickened by dense fibrous tissue, in which the vasa vasorum revealed extensive endarterial hyperplasia with considerable narrowing of their lumens. They were surrounded frequently by slight aggregations of lymphoid cells. This process continued unabated into the periaortic subepicardial tissue. The Levaditi stain was negative.

Right Aortic Cusp Through the Defective Portion The media and adventitia of the root of the aorta revealed lesions identical with those just described. The process could be seen extending downward toward the origin of the anterior flap of the mitral valve.

Superficially (corresponding to the diffuse, pearly, finely striated deposit noted in the gross specimen) there was a proliferated mass of loose, spongy, cellular tissue containing many young and mature fibroblasts, a rare thin-walled capillary was evident in it.

The ridge corresponding to the remnant of the cusp was composed of a triangular, dense, vascularized expansion from the inflamed intervalvular fibrosa. It contained irregular arrangements of elastic fibers, frequently in compact masses, occupying chiefly the periphery of the central fibrous area. Many thin-walled capillaries and a sparse number of small round cells were present.

Anterior Mitral and Posterior Aortic Cusps The posterior aortic cusp and spongy ring were normal. The annulus fibrosus and the tissue immediately behind it (fig. 4A) were moderately invaded by lymphocytes, capillaries and arterioles, the latter frequently with obliterative endarterial lesions identical with those described in the aorta. The process could be traced unchanged in degree or character through the intervalvular fibrosa and ring of the mitral valve into and along each side of the fibrous layer of the anterior leaflet (fig. 4B) for half its length. Its thickness was thereby roughly quantupled.

The normally delicate auricular layer of elastic fibers became frayed and almost lost in this region. Superficial to it was a poorly elastified, well capillarized and infiltrated fibrous tissue hyperplasia, actually wider than the original fibrosa layer. On the opposite side a similar but less vascularized proliferation of fibrous tissue rested on an intact ventricular layer of elastica and was seen to be continuous with the hyperplastic tissue noted in the region of the defective aortic cusp.

Posterior Cusp of the Mitral Valve The valve and ring were normal.

Pulmonary Valve The valve and ring were normal. The adventitia of the root of the pulmonary artery presented a picture which was identical with that in the aorta, and which undoubtedly represented an extension through the subepicardial tissue at the base of the heart.

Tricuspid Valve The ring and valve were normal. In the septum fibrosum the collagen fibers were arranged in whorls. There was a moderate degree of capillarization with sparse focal accumulations of lymphocytes. In the bundle of His, at this level, could be seen marked intimal proliferation of the arterioles, occasional lymphocytic aggregations and distinct fibrosis. The endocardium on the left aspect of the septum fibrosum was tremendously thickened by a loose, spongy fibrous tissue proliferation containing extreme numbers of fibroblasts and occasional capillaries.

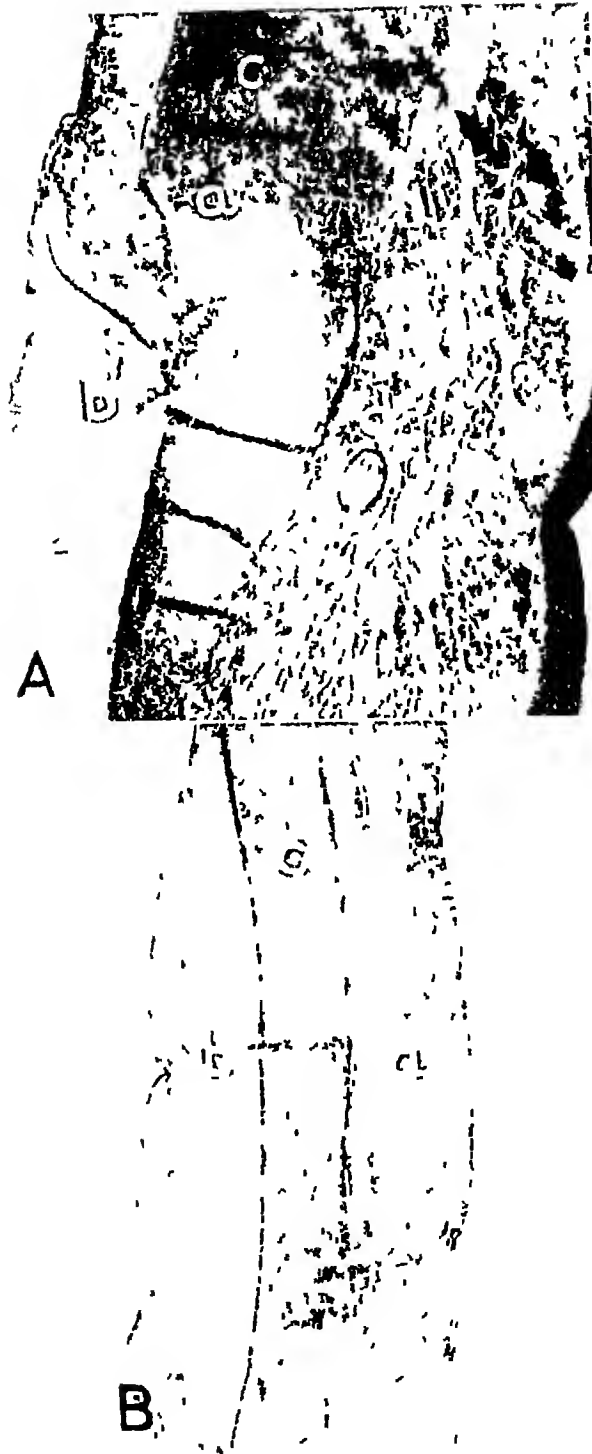


Fig 4 (case 2) —*A*, region of the annulus fibrosus (*a*) of the posterior aortic cusp. Note subaortic proliferation of avascular fibrous tissue (*b*). The inflammatory process in the root of the aorta (*c*) is continuous with that in and behind the annulus. A vessel in the latter situation is obliterated by endarteritic hyperplasia (*d*). *B*, inferior prolongation of the block of tissue from which section *A* was cut. Note the aortic leaflet of the mitral valve, showing broad hyperplastic inflammatory proliferations on the auricular (*a*) and ventricular (*b*) side of the thickened, infiltrated fibrosa layer (*c*). Both *A* and *B* show staining with Weigert's elastica stain, hematoxylin and Van Gieson's stain. Magnification, 35 mm, $\times 12$.

Left Auricle, Left Ventricle and Right Ventricle These were normal

Other Organs Other postmortem findings of importance were confluent bronchopneumonia of the middle and lower lobes of the right lung, infarction of the lower lobe of the left lung, with acute fibrinous pleuritis, bilateral hydrothorax, hydroperitoneum, edema of the lower extremities, chronic passive congestion of the liver and spleen, old infarct scar of the left kidney and thrombosis of the left subclavian vein near its mouth No lesions of syphilis were found in any of the other organs¹⁸

COMMENT ON CASE 2

The cardiovalvular lesions found in this 20 year old girl were very unusual The severely scarred aorta the destructive lesion of the aortic valve and the fibrous thickening of the anterior mitral leaflet were contiguous processes and were unquestionably inflammatory in origin

The youth of the patient (in whom the clinical history indicated a valvular defect in her eighteenth year) seems to militate against a possible syphilitic etiology and favors the likelihood of rheumatic cardiovalvular disease However, the latter may be satisfactorily excluded from the diagnosis since careful study of many sections failed to reveal any lesions suggestive of it

Microscopic examination of the lesion at the root of the aorta disclosed a very severe grade of aortitis which was recognized by available criteria to be distinctly syphilitic It is out of all proportion to the rheumatic lesions described in the aorta by Klotz¹⁹ and by Pappenheimer and Von Glahn²⁰

The destructive loss of one half of an aortic cusp suggests the possibility of bacterial endocarditis²¹ as the etiologic agent In the absence of absolute evidence to the contrary, this diagnosis cannot be definitely excluded, although it is hardly likely In addition, one must not overlook the possible etiologic significance of the paratyphoid and influenzal infections in her childhood

In searching the literature for a clue as to the nature of this valvular defect, a strikingly similar case was encountered Spalding and Von Glahn's patient,²² a man aged 31, with a positive Wassermann reaction of the blood, suffered from cardiac decompensation Sudden death was found to have been caused by a rupture of the posterior papillary muscle due to an early gummatous focus containing spirochetes A plaque of supravulval mesoartitis was found above and involving the left posterior-anterior commissure The anterior half of the left posterior

¹⁸ The brain was not examined

¹⁹ Klotz, O Tr A Am Physicians **27** 181, 1912

²⁰ Pappenheimer, A M, and Von Glahn, W C Am J Path **3** 583 1927

²¹ The bacterial stain was negative, although this does not exclude a healed stage

²² Spalding, E D, and Von Glahn, W C Bull Johns Hopkins Hosp **32** 30, 1921

aortic cusp was entirely destroyed, a small ridge indicating its previous site of attachment. The left half of the anterior cusp was also eroded but to a lesser extent.

The fibrous thickening of the anterior mitral leaflet bore several points of resemblance to the finding in case 1. Reference to the autopsy protocol and to the photomicrographs (fig 4 *A* and *B*) indicates that here again a similar inflammatory process followed the same path of extension from the base of the aorta to the anterior flap of the mitral valve and on to the endocardium over the membranous septum. However, the mitral valvular and septal lesions were less intense. In fact, the lesion in the bundle of His apparently failed to produce heart block in this case.

Despite the negative Levaditi stains and the age of the patient, the accumulated data point to syphilis as the probable cause of the lesions. The characteristic aortitis, the similarity of the aortic valvular lesion to that in Spalding and Von Glahn's² case of proved syphilis and the resemblance of the mitral valvular lesion to that in case 1 and in Friedman's⁷ and Staemmler's⁸ cases support this view. Unfortunately the patient did not live long enough for a serologic examination, the reliability of the previous examinations is uncertain.

The age of the patient suggests the possibility of congenital tertiary syphilis, which cannot be entirely ruled out. However, the rarity of authentic cases of congenital syphilitic aortitis after infancy²³ and the absence of stigmas of congenital syphilis render such a diagnosis very doubtful.

RECAPITULATION

Acquired tertiary syphilitic heart disease (exclusive of commissural aortitis and the nondiagnostic type of myocardial fibrosis) has heretofore been considered synonymous with gummatous lesions.

However, the two cases reported here presented macroscopic and histologic lesions which, when considered in connection with those in other cases recorded in the literature, are extremely suggestive of syphilis. They point to the existence of tertiary syphilitic cardiac lesions which are not distinctly gummatous. In other words, it appears that, in addition to diffuse and localized gummatous lesions (described in a previous report¹), a third type of syphilitic involvement can be recognized in which coagulation necrosis and giant cells are absent.

This lesion is apparently very rare. Its gross appearance is that of a fairly well circumscribed, densely sclerotic, whitish, cartilaginous mass. In a valve it produces marked thickening of the structure. The histologic picture is one of dense irregular or whorled connective tissue formation. Valvular elastic lamellae are reduplicated and frayed. The

entire lesion is apt to be extensively vascularized, capillaries and arterioles with obliterative endarterial lesions are found. These are usually surrounded by focal accumulations of lymphocytes, plasma cells and large mononuclear cells. Diffuse cellular infiltrations are likewise present. In the anterior leaflet of the mitral valve, the process arises by extension from syphilitic disease at the root of the aorta.

In summary the picture is that of a well scarred granuloma in which necrotic foci have disappeared. The fact that coagulation necrosis is absent should not exclude the diagnosis of syphilis any more than it does in the case of syphilitic aortitis. The lesion in the mitral valve in the cases of Friedman⁷ and Staemmler⁸ belongs to this type of granulomatous involvement differing however by possessing areas of coagulation necrosis and less prominent fibrosis, i. e., a more active type of granuloma.

SUMMARY

Two extremely unusual instances of cardiovalvular disease are reported in which the accumulated evidence points to a presumptive diagnosis of acquired tertiary syphilitic heart disease.

The lesion encountered represents a well scarred granuloma most probably syphilitic.

The question is raised as to whether the typical gummatous lesion of the heart (apart from commissural aortic syphilis and the non-diagnostic syphilitic myofibrosis) constitutes the *sine qua non* of cardiac syphilis.

VASCULAR LESIONS IN SURGICALLY EXCISED STOMACHS

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The frequent observation of vascular lesions in surgically excised stomachs in these laboratories led to the present study of such lesions in relation to gastric and duodenal ulcer. Although much has been written concerning gastric arteriosclerosis, its importance is probably much underestimated. A few workers have held that vascular disease is the primary factor in the causation of peptic ulcer. There are numerous opponents to this view who not only refuse to accept it in its entirety but have set vascular disturbances entirely aside in theoretical considerations of the pathogenesis of peptic ulcer. Admittedly there is no proof that vascular lesions are the main factor in the production of gastric ulcer. However, it seems not unlikely that arteriosclerosis may play a rôle in the localization and persistence of ulcer. With this in mind a careful systematic study of the arterial changes in a series of resected gastric specimens was undertaken. Attention was directed particularly to the nature and distribution of the vascular changes. As a result of the study certain conclusions have been drawn in regard to their etiology and their possible significance in the pathogenesis of peptic ulcer.

Arteriosclerosis of abdominal vessels was mentioned by von Rokitsansky and Lebert as early as 1852. In 1853 Virchow¹ postulated a relationship between gastric ulcer and arteriosclerosis, but it was not until 1884 that pathologic studies of gastric arteriosclerosis, per se, began to appear in the literature. In that year Gallard² published a report on two cases of gastric arteriosclerosis, with autopsy. One was that of a young man 28 years of age, who had experienced epigastric discomfort and hematemesis. Post mortem there was found a small mucosal erosion, in the base of which lay a tiny artery with aneurysmal dilatation. In the other case, that of a man 51 years of age, autopsy disclosed a similar gastric mucosal erosion, also presenting at its base a small aneurysm of a submucosal artery.

From this time on, case reports of gastric arteriosclerosis cropped up sporadically in the literature. In 1908, Lewin,³ after reviewing the literature on gastric arteriosclerosis then extant, and adding two cases of his own, concluded "1 It is possible to have severe arteriosclerosis of the stomach with only slight arterio-

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1 Virchow, quoted by Ophuls, W. Arch Int Med **11** 469, 1913

2 Gallard, quoted by Hamburger, W. W. Deutsches Arch f klin Med **97** 49, 1909

3 Lewin, A. M. Arch f Verdauungskr **14** 114 1908

sclerosis of the aorta and other vessels 2 Arteriosclerosis of the stomach may lead to multiple aneurysms 3 Arteriosclerosis of the stomach may occur in very young people" Buday,⁴ in the same year, reported the autopsy in a case of widespread, marked sclerosis of gastric arteries and moderate general arteriosclerosis

Hamburger⁵ in 1909 collected a number of cases with autopsy, beginning with that of Gallard in 1884 He was able to glean twelve case reports from the literature In four of these an aneurysmal dilatation of a submucosal artery was found in the base of a mucosal erosion Hamburger noted that in only six of the twelve cases had the tissues been subjected to microscopic investigation, and called attention to the case of Carrier in which no gross lesions could be demonstrated He noted, moreover, that the ages of the patients ranged from 28 to 79 years, and that in all there was a tendency to general arteriosclerosis

Hamburger's investigations involved the study of gastric arteries in cadavers of persons of both sexes and of widely varying ages He compared the arteries of the stomach with arteries elsewhere The relationship between changes in the arteries and changes in the gastric mucosa was studied He presented ten cases, five in men and five in women He found that there were definite changes in the gastric arteries of the six patients who were under 43 years of age Of the four older patients, two showed no evidence of sclerosis of the gastric arteries, although there was considerable general arteriosclerosis Of the entire number, six showed slight sclerotic changes within the arteries of the stomach, one presented moderate change and one marked change Hamburger encountered no aneurysmal dilatations of small submucosal vessels such as had been described by Gallard, Sachs and Hirschfeld His cases presented an extreme variability of the arterial lesions in the stomach as to distribution, extent and severity In three of the cases, the gastric arteries were not involved uniformly, certain branches being affected while others remained completely free He remarked that there was no rhyme or reason to the distribution of sclerosis in gastric arteries Regarding the relationship between general arteriosclerosis and sclerosis of the gastric vessels, Hamburger stated "From the condition of the larger vessels one cannot come to a conclusion regarding the state of those of the stomach" Finally, he observed that the vessels along the lesser curvature of the gastric wall were most often affected

Ophuls,⁶ in a paper concerned mainly with the consideration of the etiology and pathogenesis of peptic ulcer, tabulated eighteen cases of chronic ulcer of the stomach associated with general arteriosclerosis which had come to autopsy The gastric arteries were examined in five of the cases, arteriosclerosis was noted either in the arteries in the base of the ulcer or in vessels supplying the region in which the ulcer occurred In the same communication, he reported four cases of chronic peptic ulcer in young persons The ages ranged from 24 to 39 years He was able to find gastric arteriosclerosis, associated with little or no general arteriosclerosis, in each one of this group Regarding the origin of the local arteriosclerosis, Ophuls stated " I am inclined to believe that in such instances also one is dealing with a local primary disease of the arteries like that which nobody denies to exist in cases of spontaneous gangrene of the leg in young individuals The one strong reason in favor of this view is that the disease in the arteries in these cases also usually extends a good distance beyond the base of the ulcer "

4 Buday, K V Beitr z path Anat u z allg Path **44** 327, 1908

5 Hamburger, W W Deutsches Arch f klin Med **97** 49, 1909

6 Ophuls, W Arch Int Med **11** 469, 1913

Zeek and Phair⁷ reported three cases that had come to autopsy, in each of which diffuse sclerosis of small vessels was encountered within the gastro-enteric tract. In two of the cases there was massive gangrene of the stomach and intestines. In the third there was extensive ulceration of the enteric tract without involvement of the stomach. Microscopic examination in each case revealed marked intimal thickening of the submucosal arteries, with ulceration and inflammation of the areas of mucosa supplied by them. There was evidence of general arteriosclerosis in all three patients.

From the preceding brief review of the literature it is evident that much serious thought and study have been given to the problem of gastric arteriosclerosis, particularly from a pathologic point of view. A few of the points mentioned, I consider, cannot be overstressed. The suggestion of Ophuls that gastric arteriosclerosis is a local primary disease of the arteries is of importance. It may be seen from the work of both Buday and Hamburger not only that gastric arteriosclerosis is not necessarily associated with general arteriosclerosis, but that general arteriosclerosis, or sclerosis of great vessels, may exist without con-

TABLE 1—*Conditions Represented in Specimens*

Diagnosis	Number of Cases
Gastric ulcer	7
Duodenal ulcer	13
Secondary (previous operation had been performed)	8
Gastritis (symptoms of ulcer but no ulcer could be demonstrated clinically or pathologically)	2
Total	30

comitant sclerosis of the arteries of the stomach. The finding by Hamburger that the vessels along the lesser curvature of the gastric wall were oftenest affected is of interest. The frequent mention by various writers of involvement of submucosal vessels, particularly in relation to mucosal ulceration, is worthy of note. It seems fairly clear from the descriptions of the nature of the vascular changes that chronic nodular endarteritis was the lesion usually encountered, and that the aneurysmal dilatations of submucosal arteries described by Gallard, Sachs and Hirschfeld fit into this category.

MATERIAL

A series of thirty surgically resected portions of stomach⁸ received in this laboratory from April 1933 to April 1934 was studied. Only those specimens removed because of malignant disease were excluded. The patients from whom the specimens were excised ranged from 23 to 63 years of age. The average age was 42 years. The sex ratio was roughly 2:1, as 21 of the specimens were from men and 9 from women. The specimens were grouped as shown in table 1.

7 Zeek, P., and Phair, J. J. *Am J M Sc* **181** 548, 1931.

8 From the Surgical Service of the Toronto General Hospital.

The ulcer was present within the excised specimen in seven of the cases of duodenal ulcer. In the remainder of this group the ulcer was knowingly left behind at operation (Polya operation). Of the eight ulcers listed as secondary, seven had been diagnosed as duodenal ulcer prior to operation or at a previous operation. Each may or may not have been present or active at the time of secondary operation. Stomal ulcers were identified pathologically in the specimens from four of these cases.

The specimens were pinned out on cardboard and fixed in solution of formaldehyde U S P (1:10) as soon as possible following removal, to eliminate the

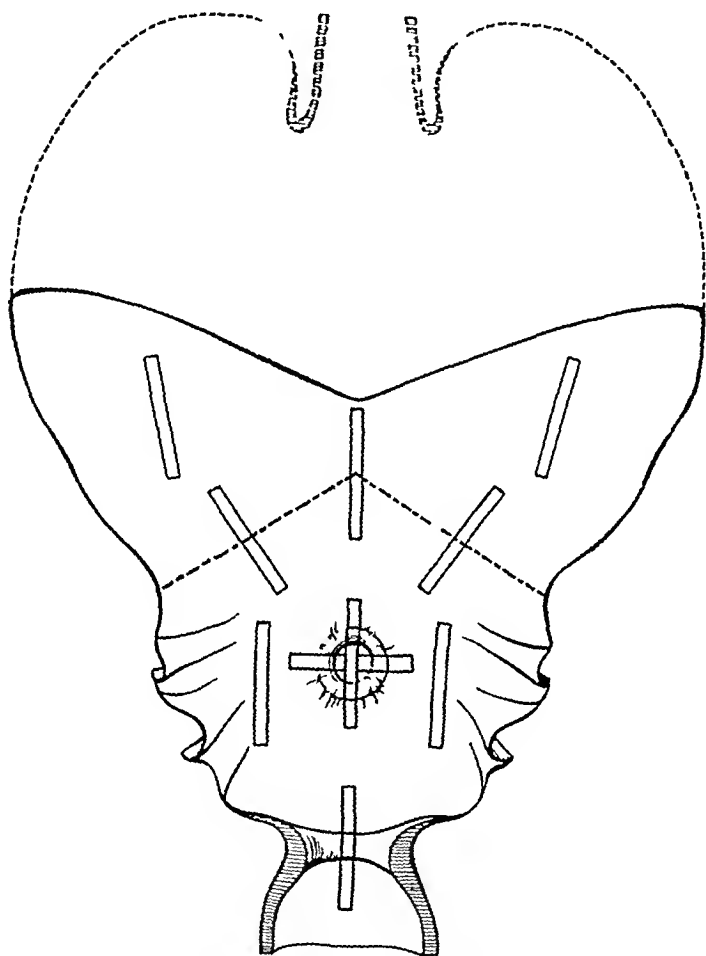


Fig 1—A diagram showing the plan followed in the choice of blocks for microscopic examination

factor of autodigestion. A rough sketch was made of each specimen. Blocks for microscopic study were taken in every case, adhering as closely as possible to the diagram (fig 1). The site from which each block had been taken was outlined on the drawing of the specimen. Paraffin sections were made and stained with hematoxylin and eosin. In a few instances either elastin H or Weigert's elastic tissue stain was used in addition.

OBSERVATIONS

The results are tabulated. The tables are self-explanatory and deal particularly with the degree of gastritis and the distribution and degree

of arteriosclerosis in each specimen. The cases have been divided into four groups according to the indication for resection. An analysis of each group is given, followed by an analysis in which the cases are divided into two groups according to age. Finally, the nature of the gastritis and endarteritis encountered in the entire series is described.

Analysis of Table 2—In each of the seven specimens, marked intimal thickening of arteries in the vicinity of the ulcer was noted. Of more interest, however, was the constant occurrence of arteriosclerosis at

TABLE 2—Group of Seven Specimens Resected for Gastric Ulcer

No.	Age	Sex	Description of Specimen	Degree of Gastritis or Duodenitis	Distribution and Degree of Arteriosclerosis
4	45	M	Pylorus with portions of duodenum and fundus, prepyloric gastric ulcer	Marked	Marked intimal thickening of arteries within floor of ulcer marked in several submucosal arteries some distance from edge of ulcer
6	35	M	Pylorus with portion of fundus, pyloric ulcer, high on lesser curvature	Marked in pylorus, slight in fundus	Marked intimal thickening in vessels in floor of ulcer and in submucosal arteries of pylorus near greater curvature
22	43	M	Pylorus with portions of fundus and duodenum, pyloric ulcer high on lesser curvature	Marked	Marked intimal thickening in floor of ulcer and in number of submucosal arteries in fundus arteriosclerosis present as distant as 7 cm. from edge of ulcer
24	50	M	Pylorus with portion of fundus and duodenum, small prepyloric gastric ulcer	Marked in pylorus, slight in fundus	Slight to marked intimal thickening in a number of submucosal arteries lesions not more marked in or near ulcer
25	40	F	Pylorus with portions of fundus and duodenum, penetrating pyloric ulcer high on lesser curvature, hour glass deformity	Marked in pylorus, moderate in fundus	Marked intimal thickening in base of ulcer and within 2 cm. of edge of ulcer in all directions marked in a number of small arteries in submucosa, muscularis and subserosa in all blocks
27	46	F	Pylorus with portions of fundus and duodenum, gastric ulcer high on lesser curvature, hour glass deformity	Marked	Marked intimal thickening within base of ulcer, as well as in a number of submucosal arteries in duodenum, pylorus and fundus, away from ulcer
29	40	M	Pylorus with portions of fundus and duodenum, prepyloric gastric ulcer	Marked in pylorus, slight in fundus	Marked intimal thickening of vessels in and near ulcer, moderate or marked in a few subserosal vessels of fundus, slight in a few submucosal vessels of fundus

some distance from the edge of the ulcer. This was noted particularly in submucosal arteries, whether of the pylorus or of the fundus. The distribution of the arterial lesions was always patchy in that two or three submucosal arteries in a certain block revealed marked intimal thickening while possibly twenty or more similar vessels in the same block did not present the slightest evidence of pathologic change. The gastritis was marked in the pyloric region in each instance. In four of the seven specimens, however, there was only slight or moderate gastritis in the fundic area. The duodenum, in each instance in which it was present, showed marked duodenitis. The outstanding finding in this

series was, I believe, the occurrence in every case of marked intimal thickening of a number of submucosal arteries not in the vicinity of the ulcer

TABLE 3—*Group of Thirteen Specimens Resected for Duodenal Ulcer*

No	Age	Sex	Description of Specimen	Degree of Gastritis or Duodenitis	Distribution and Degree of Arteriosclerosis
1	50	M	Pylorus with portions of duodenum and fundus (ulcer not included)	Moderate	Slight to moderate intimal thickening, in several submucosal arteries of pylorus, marked in submucosal arteries of fundus midway between greater and lesser curvatures
2	56	F	Pylorus with portions of duodenum and fundus, duodenal ulcer	Moderate	Slight intimal thickening in a few submucosal arteries near pyloro-fundic junction, none in base of ulcer
3	59	M	Cuff of fundus (ulcer not included)	Slight	None
7	49	F	Pylorus with portion of duodenum, duodenal ulcer	Marked	Marked intimal thickening of arteries near and in base of ulcer, also in subserosal and submucosal vessels high in pylorus on posterior wall near lesser curvature
9	32	M	Pylorus with portion of fundus (ulcer not included)	Marked	Slight intimal thickening in a few submucosal arteries, in nearly all of blocks
10	31	M	Pylorus with portion of duodenum (ulcer not included)	Marked	Marked intimal thickening in number of submucosal arteries of duodenum, similar change in only a few arteries of pylorus
11	29	F	Pylorus with portions of duodenum and fundus (ulcer not included)	Moderate	Slight to moderate intimal thickening in a few submucosal arteries in all areas
13	33	M	Pylorus with portion of duodenum, duodenal ulcer	Marked	Marked intimal thickening in arteries at base of ulcer, slight in a few submucosal arteries in other sections
16	58	F	Pylorus with portion of duodenum, superficial duodenal ulcer	Moderate	Marked intimal thickening of submucosal arteries in duodenum at ulcer, and at pyloric ring, moderate intimal thickening of submucosal vessels high in pylorus
20	30	F	Pylorus with portions of duodenum and fundus, duodenal ulcer	Marked in pylorus, slight in fundus	Moderate or marked intimal thickening of submucosal vessels in duodenum and distal portion of pylorus
21	46	M	Pylorus with portion of fundus (ulcer not included)	Marked	Marked intimal thickening in many submucosal branches in pylorus, also in occasional branches in muscularis and subserosa, moderate intimal thickening in submucosal arteries of fundus
26	35	M	Pylorus with portions of fundus and duodenum, duodenal ulcer	Marked	Marked intimal thickening of vessels in vicinity of ulcer, also in submucosal vessels of pylorus and lower portion of fundus
34	38	M	Pylorus with portions of duodenum and fundus, duodenal ulcer	Moderate in pylorus, slight in fundus	Marked intimal thickening of a few tiny vessels in vicinity of ulcer, slight in a few submucosal arteries of pylorus, none in fundus

Analysis of Table 3—The ulcer was present in seven of the specimens. In six of these there was marked intimal thickening of certain arteries in the vicinity of the ulcer. No arteriosclerosis was noted however in the base of the ulcer in the remaining one although there was slight intimal thickening of a few submucosal arteries high in the pylorus. In these seven cases study of arteries in sections away from

the vicinity of the ulcer revealed slight intimal thickening in three, moderate in two and marked in two. In six instances this involvement was of submucosal vessels alone. The other specimen presented marked intimal thickening in both submucosal and subserosal vessels away from the ulcer.

Of the six specimens received in which no ulcer was present, five showed varying degrees of endarteritis and one presented none. Judging from the propinquity of the vascular lesions to the distal edge of the specimen, I believe that in two of the cases the intimal thickening was most marked near the ulcer. In three specimens, however, this did not appear to be the case. Two of these showed slight or moderate intimal thickening of submucosal arteries in all areas. In the third there was marked intimal thickening of several submucosal arteries of the fundus, midway between the greater and the lesser curvature, in addition to marked intimal thickening of arteries in the pylorus.

Of the entire thirteen specimens, gastritis or duodenitis was slight in one, moderate in five and marked in seven. In two instances, the gastritis was less marked in the fundic region. In those specimens which included a portion of duodenum, the degree of duodenitis corresponded fairly well with that of the gastritis. As to the relationship between the gastritis and the endarteritis, little can be said at this point save that in the specimen which showed only slight gastritis, no arterial lesions were found. In summing up, it seems worthy of note that in this group involvement of submucosal vessels particularly was observed frequently at some distance from the vicinity of the ulcer. The absence of arterial change in the base of the ulcer in one case is also of interest.

Analysis of Table 4—Of the eight specimens in this group, four presented stomal ulcers. In two of the latter, intimal thickening of submucosal vessels was more marked in the region of the ulcer. In the other two, arteriosclerosis was no more severe in the vicinity of the ulcer than at a distance. Of the four specimens in which no stomal ulcer was found, one presented no endarteritis. Of the remaining three, two presented marked intimal thickening of submucosal arteries in all areas. The third, in which but a small section of gastric wall was included, presented marked intimal thickening of a few submucosal vessels within its limits.

Gastritis, duodenitis or jejunitis was marked in five cases and moderate in three. I think it worthy of emphasis that in this group, as well as in the two preceding groups, there was evidence of chronic endarteritis distant from any ulcerative process.

Analysis of Table 5—Although the gastritis was marked in both of these specimens the endarteritis was of slight degree. The submucosal arteries were involved.

Analysis According to Age—Sixteen of the patients fell into a group ranging from 20 to 40 years of age. The remainder (fourteen) comprised a group from 41 to 63 years of age. The relative incidence of

TABLE 4—Group of Eight Specimens Resected Secondarily

No.	Age	Sex	Description of Specimen	Degree of Gastritis, Duodenitis or Jejunitis	Distribution and Degree of Arteriosclerosis
5	63	M	Pylorus with portions of duodenum, fundus and jejunum, gastro enterostomy stoma	Marked	Marked intimal thickening of muscular arteries along greater curvature, near pylorofundic junction, moderate or marked in scattered vessels elsewhere, sclerosis is not more marked in region of stoma
8	23	F	Pylorus with portions of duodenum and fundus, gastro enterostomy stoma with superficial erosion	Marked	Moderate intimal thickening in one subserous vessel of pylorus on greater curvature, slight intimal thickening of several submucosal branches of pylorus
12	44	M	Pylorus with portions of fundus and jejunum, gastro enterostomy stoma	Marked	Marked intimal thickening in a number of submucosal arteries in all portions of specimen
14	23	F	Cuff of fundus	Moderate	None
15	34	M	Small ring of gastric wall (fundus), with attached length of jejunum	Moderate	Marked intimal thickening of several submucosal arteries in stomach (fundus)
18	36	M	Pylorus with portion of fundus, gastro enterostomy stoma, stomal ulcer	Marked	Marked intimal thickening of arteries in vicinity of ulcer, none in remainder of fundus, slight in several submucosal vessels of pylorus
23	38	M	Pylorus with portions of duodenum, fundus and jejunum, gastro enterostomy stoma, stomal ulcer	Moderate	Slight to moderate intimal thickening of submucosal muscular and subserosal arteries throughout pylorus and fundus, arteriosclerosis is not more marked in vicinity of ulcer
28	36	F	Pylorus with portions of fundus and jejunum, gastro enterostomy stoma with superficial erosion	Marked	Marked intimal thickening of submucosal arteries in jejunum and fundus near stoma, slight to moderate in submucosa of pylorus, moderate to marked in fundus along lesser curvature

TABLE 5—Group of Two Specimens Resected for Gastritis

No.	Age	Sex	Description of Specimen	Degree of Gastritis or Duodenitis	Distribution and Degree of Arteriosclerosis
17	40	M	Pylorus and portion of fundus	Marked	Slight intimal thickening in a number of submucosal arteries in both fundus and pylorus
19	58	M	Pylorus and portion of duodenum	Marked	Slight intimal thickening in a few submucosal arteries near pyloric ring

gastric arteriosclerosis was interesting. Only the arteriosclerosis noted outside of the vicinity of the ulcer was considered in this analysis. Of the first group (from 20 to 40 years), six presented marked arteriosclerosis, three moderate, six slight and one none. Of the second group, eight presented marked arteriosclerosis, three moderate, two slight and one none.

Nature of the Gastritis—As indicated in the tables, nearly every stomach presented a well marked chronic inflammatory change within its wall. This "chronic follicular gastritis" or "chronic gastritis" was identical with that described by Johnston⁹ in these laboratories in an earlier series of cases associated with peptic ulcer. The histologic features of the gastritis in this series can be outlined briefly. The mucous membrane was edematous and heavily infiltrated by polymorphonuclear leukocytes, eosinophils, lymphocytes and plasma cells. Similar cells were often scattered sparsely throughout the submucous and muscular coats. The pylorus presented in many instances a more severe degree

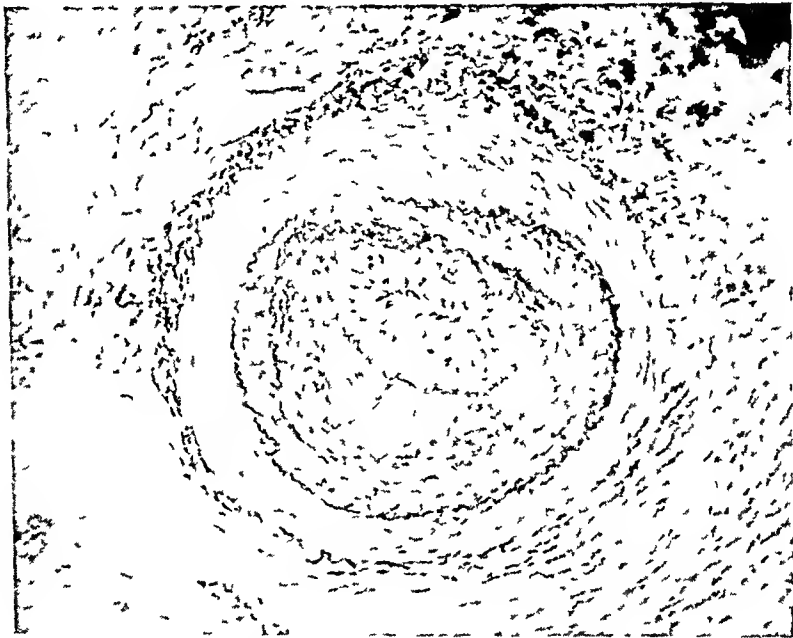


Fig 2—A nearly obliterated artery in the floor of a gastric ulcer, elastin H stain. Note the reduplication of the internal elastic lamina, the endothelial proliferation and the inflammatory reaction in the adventitial and perivascular tissue, $\times 80$.

of inflammatory change than the fundus. Hypertrophied lymph follicles were plentiful within the pyloric mucosa of many specimens.

Nature of the Arterial Changes—The sclerotic changes within the affected arteries were essentially proliferative and involved the intima (chronic nodular endarteritis). Splitting or reduplication of the internal elastic lamina was found in many instances (fig 2). The submucosal arteries, which were most frequently involved, showed varying degrees of thickening and sometimes marked narrowing of the lumen, even when located far from the vicinity of the ulcer (fig 3). Although a degen-

9 Johnston, C. R. K. Surg, Gynec & Obst 58 614, 1934

erative intimal change had occurred in a few of the involved vessels, it appeared to represent a secondary phase. Medial thickening was not common and when present was slight. No degenerative medial change could be detected. Periarterial or adventitial inflammation was demonstrable within a small number of the affected arteries. This inflammatory reaction was cellular and nonproliferative. The intimal or medial coats were in no instance invaded by inflammatory cells.

COMMENT

A general discussion of the etiology of arteriosclerosis is not within the scope of this communication. Vasomotor or angioneurotic dis-

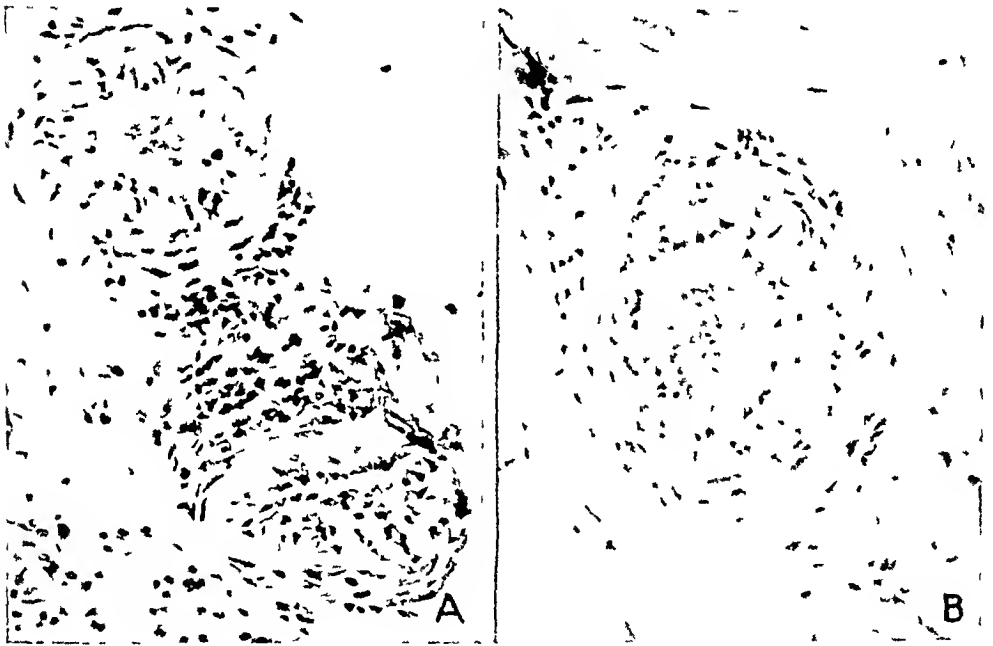


Fig. 3—*A*, submucosal arteries at the fundus some distance from the ulcer in the same case as figure 2, $\times 160$. *B*, a submucosal pyloric artery some distance from an ulcer, showing slight infiltration by inflammatory cells in the adventitial tissue, $\times 160$.

turbances have been suggested as the cause of arteriosclerosis in general and of gastric arteriosclerosis in particular (Held¹⁰). I believe, however, that there is more evidence experimental and otherwise that the arteriosclerosis encountered in the present study is of infective origin. The morphologic nature of the arterial changes is entirely compatible with this view. Klotz¹¹ showed experimentally that streptococcal or typhoid infections produced intimal proliferation, with splitting of the internal elastic lamina in the ascending aorta and pulmonary artery of

10. Held, I. W. *M. J. & Rec.* 122: 6, 1925.

the rabbit. Attention has been called to the endarteritis frequently present in arteries situated within infective foci by both Klotz¹¹ and Andrewes¹². The latter pointed out that an artery may become involved either by deposition of bacteria or similar material directly on the intimal coat or by invasion through the vasa vasorum of the adventitia or media. It is not inconceivable that in the series of stomachs under consideration the endarteritis is closely related to the gastritis shown to have been present in all. The frequent involvement of the submucosal vessels favors this view in that the gastritis is always most marked within the mucosa and submucosa. The occasional appearance of an active cellular inflammatory reaction about one of the affected vessels lends further strength to the belief.

The tremendous quantity of literature concerning the etiology and pathogenesis of peptic ulcer has been ably summarized by Hurst and Stewart¹³. Only a few of the present-day theories can be mentioned in this discussion. Many observers, including Vichow, Ophuls and Hauser, have held that gastric or duodenal ulcers arise as a result of local vascular disturbances. According to this view, the ulcer is primarily infarctive. Opponents of this theory apparently do not admit the existence of primary gastric arteriosclerosis. At present much importance is attached to the experimental work of Mann and Williamson¹⁴, who were able to produce peptic ulcers in dogs by the institution of surgical duodenal drainage. Those who prefer the biochemical theory of ulcer causation are afforded a measure of experimental proof by this work. Lately, the view has been advanced that peptic ulcer is but a phase in the course of gastritis or duodenitis (Konjetzny,¹⁵ Faber¹⁶). The almost constant presence of gastritis in cases of peptic ulcer cannot be denied.

The health of any member or physiologic unit of the human body is dependent on the integrity of its circulation. Functional disturbances or organic lesions may occur if the local blood supply becomes, for any reason, inadequate. The infliction of slight injuries on poorly vascularized portions of the human integument is frequently followed by the development of chronic persisting ulcers. Examples are varicose ulcers

11 (a) Klotz, O. *Brit. M. J.* **2** 1767, 1906, (b) *Boston M. & S. J.* **156** 267, 1907.

12 Andrewes, F. W. Report on Arterial Degeneration, Rep. M. Off. Local Gov. Bd., London, 1913, Appendix B, no. 1, Second Report on Arterial Degeneration, Rep. M. Off. Local Gov. Bd., London, 1913-1914, Appendix B, no. 1, p. 151.

13 Hurst, A. F., and Stewart, M. J. *Gastric and Duodenal Ulcer*, New York, Oxford University Press, 1929.

14 Mann, F. C., and Williamson, C. S. *Ann. Surg.* **77** 409, 1923. Mann, F. C. *S. Clin. North America* **5** 753, 1925.

15 Konjetzny, quoted by Faber¹⁶.

16 Faber, K. *Lancet* **2** 901, 1927.

and ulcers of poorly nourished skin grafts. The exciting factor of insult or trauma cannot, of course, be discounted. An analogy may exist in the instance of chronic gastric or duodenal ulcer in which highly acid gastric juice serves as the exciting factor and local lowering of resistance secondary to arteriosclerosis, as the underlying predisposing anatomic factor. Thus, I believe the part played by arteriosclerosis in the pathogenesis of gastric or duodenal ulcer to be contributory or secondary. Even though it is granted that irritative effects of acid gastric juice are most important, the localization of an ulcer to a certain area of the mucous membrane cannot be satisfactorily explained except by the existence of a narrowing of the lumen of certain submucosal vessels. As shown by Reeves¹⁷ the submucosal arteries of the stomach and duodenum become end-arteries as they enter the mucosa. Each of these terminal vessels supplies an area of mucosa measuring approximately 2.5 mm in diameter. Reeves found that normally the submucosal arteries along the lesser curvature of the stomach and first inch of the duodenum are more tortuous, narrower and less numerous than elsewhere. Nearly all ulcers arise in one or the other of these locations. Attempts to produce ulcer experimentally by causing embolism or thrombosis of gastric arteries have been successful only when the injected substance became lodged in a submucosal artery (Schridde¹⁸). The frequent occurrence of mucosal erosions and superficial ulcers in association with gastritis is well known. It seems likely that a mucosal erosion occurring in an area where local resistance has been lowered by vascular disease is more liable to persist and become chronic than one in an area the circulation of which is undisturbed.

CONCLUSIONS

A local arteriosclerosis is commonly present within stomachs resected surgically for gastric, duodenal or stomal ulcer.

The arteriosclerosis is patchy in distribution and affects chiefly the submucosal vessels. It is not necessarily confined to the base or floor of the ulcer, but may be present at some distance from the ulcer.

There is apparently little relation between this local arteriosclerosis and age.

The arteriosclerosis found is essentially a proliferative intimal lesion (chronic nodular endarteritis).

This endarteritis may occur as a part of the gastritis shown to be uniformly present.

It is suggested that gastric arteriosclerosis may, as an underlying anatomic factor, play an important part in the localization and persistence of gastric or duodenal ulcer.

17 Reeves, T. B. Surg., Gynec. & Obst. **30** 374, 1920.

18 Schridde, quoted by Stewart, M. J. Brit. M. J. **2** 955, 1923.

EARLY LESIONS FOLLOWING INTRAVENOUS ADMINISTRATION OF A FILTRABLE STAPHYLOCOCCUS TOXIN

A STUDY ON THE DOG AND RABBIT

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DURHAM, N C

Lesions produced by staphylococcus toxin have been observed in laboratory animals since de Christmas¹ first injected toxin into the anterior chamber of the rabbit's eye in 1888. Van de Velde,² von Lingelsheim,³ Mosny and Marcono⁴ and Moise⁵ were pioneers in this field of investigation. In the early part of the twentieth century Neisser and Levaditi,⁶ Neisser and Wechsberg⁷ and Neisser and Lipstein⁸ made many contributions to the subject. Recently Nicolle and Cesari,⁹ Russ,¹⁰ Parker,¹¹ Burnet,¹² Kellaway and his associates,¹³ Gross,¹⁴ Burky,¹⁵ Forssman,¹⁶ Dolman¹⁷ Borthwick,¹⁸ Rigdon and his asso-

From the Department of Pathology, Duke University School of Medicine

1 de Christmas, J. *Ann Inst Pasteur* **2** 469, 1888

2 Van de Velde, H. *Cellule* **10** 403, 1894

3 von Lingelsheim, W. *Ätiologie und Therapie der Staphylokokkeninfektionen*, Berlin, Urban & Schwarzenberg, 1900

4 Mosny and Marcono. *Compt rend Acad d sc* **2** 962, 1894

5 Morse, J L. *J Exper Med* **1** 613, 1896

6 Neisser, M., and Levaditi, C. *Action de la toxine staphylococcique sur le rein*, *Compt rend cong internat de med (Sect de path gen et de path exper)*, 1900, p 475

7 Neisser, M., and Wechsberg, F. *Ztschr f Hyg u Infektionskr* **36** 299, 1901

8 Neisser, M., and Lipstein, A. *Die Staphylokokken*, in Kolle, W., and Wassermann, A. *Handbuch der pathogenen Microorganismen*, ed 2, Jena, Gustav Fischer, 1908, vol 3, p 105

9 Nicolle, M., and Cesari, E. *Ann Inst Pasteur* **28** 219, 1914

10 Russ, V K. *Ztschr f exper Path u Therap* **18** 220, 1916

11 Parker, Julia T. *J Exper Med* **40** 761, 1924

12 Burnet, F M. *J Path & Bact* **32** 717, 1929

13 Kellaway, C H., Burnet, F M., and Williams, F Eleanor. *J Bact & Path* **33** 889, 1930

14 Gross, Hans. *Ztschr f Immunitätsforsch u exper Therap* **73** 14, 1931

15 Burky, Earl L. *J Immunol* **24** 115, 1933

16 Forssman, J. *Acta path et microbiol Scandinav (supp)* **11** 202, 1932

17 Dolman, C E. *Canad Pub Health J* **23** 125, 1932

18 Borthwick, G R. *Brit J Exper Path* **14** 236, 1933

ciates¹⁹ and Von Glahn and Weld²⁰ have contributed to the study of staphylococcus toxin

Many investigators consider the lesions in the tissues as due to specific fractions in the staphylococcus toxin. Since no one has made a study of all the lesions in any one animal after the intravenous administration of the toxin it is the purpose of this paper (1) to describe the early lesions occurring in the dog and rabbit and to show that frequently all the lesions occur in the same animal and (2) to discuss the pathogenesis of the lesions

METHODS AND MATERIALS

Five adult dogs and forty-three adult rabbits were used in this study. The toxin was prepared from a hemolytic strain of *Staphylococcus aureus* by the method described by Parker, Hopkins and Gunther²¹ with few modifications. The dogs were given a single intravenous injection of the toxin, and the rabbits were given one or more intravenous injections at intervals of from thirty minutes to twenty-four hours. A complete autopsy was made usually immediately after death, and the tissues were fixed in Zenker's fluid to which a solution of formaldehyde had been added and in a solution of formaldehyde.

LESIONS IN THE DOG

Blood was present in the abdominal cavity of each of the five dogs. The quantity of blood varied from a few cubic centimeters to 500 cc. No rupture of a blood vessel could be demonstrated. There was no fluid or blood in the pleural, pericardial or cranial cavity. The viscera were always in their normal position.

Hemorrhagic areas were present in the diaphragm of four of the five dogs. They occurred in the muscle and beneath the parietal layer of peritoneum and pleura. The largest amount of blood was always found beneath the pleural surface.

In some of the dogs there was a questionable dilatation of the heart. However, this dilatation was not constant in this group of animals. A varying number of petechiae were found in the epicardium, myocardium or endocardium, frequently petechiae were found in all three locations.

Hemorrhagic areas were present in the pleura and in the parenchymatous tissue of the lungs. In one dog the capillaries were dilated throughout the interstitial tissue. The hemorrhagic areas were located in the alveoli and interstitial tissue and about the blood vessels. There were collections of red blood cells in some of the bronchi.

The spleen was usually purplish brown and moderately swollen. The sinusoids were greatly dilated and filled with red blood cells.

The liver was frequently the same color as the spleen, and when it was sectioned a large amount of blood escaped. In a detailed study of the hepatic lobule the cells were found to be swollen, and often they contained small vacuoles. The sinusoids were dilated and filled with blood. A few necrotic foci were present, these were surrounded by red blood cells, polymorphonuclear leukocytes, mononuclear wandering cells or fragmented hepatic cells. The most conspicuous lesion

19 Rigdon, R. H., Joyner, A. L., and Ricketts, E. T. *Am J Path* **10** 425, 1934

20 Von Glahn, William C., and Weld, Julia T. *J Exper Med* **61** 1, 1935

21 Parker, Julia T., Hopkins, J. G., and Gunther, A. *Proc Soc Exper Biol & Med* **23** 344, 1925

in the liver was an accumulation of red blood cells about the hepatic arteries and veins. In some instances it was difficult to demonstrate the entire wall of the blood vessels, as the cells in the lumen were continuous with those about the periphery.

In some of the dogs the wall of the gallbladder was edematous and hemorrhagic. The accumulation of red blood cells and the edema were most pronounced in the fibro-elastic tissue in the periphery of the wall. The mucosa of the gallbladder and ducts was grossly normal. There were no stones or obstruction in the biliary passages.

Blood was always present in some portion of the gastro-intestinal tract. The largest quantity was usually found in the ileum. Small hemorrhagic areas were present in the mucosa (fig 1). In addition, the epithelial cells lining the glands



Fig 1—Photograph of hemorrhagic areas in the mucosa of the small and large intestines of a dog that received 10 cc of staphylococcus toxin intravenously and died one and a half hours later. The hemorrhage is usually restricted to the folds of the mucosa in the colon.

and crypts throughout the gastro-intestinal tract showed all stages of degeneration from simple cloudy swelling to complete degeneration (fig 2).

The kidneys were normal in size, and the cortical surfaces were smooth. In the animals that died a short time after receiving the toxin the capillaries in the glomerular tufts were dilated and filled with red blood cells. In the dogs that lived for a longer time the endothelial cells and the epithelial cells in the glomeruli and tubules were swollen and granular, and the renal epithelial cells sometimes contained hyaline droplets. Albumin was present in the capsular spaces and in the lumens of the renal tubules in some of the dogs. Essentially every capsular space and the lumen of every tubule was filled with albumin in one dog that lived for six hours after receiving the toxin. In this animal the glomerular tufts

appeared to be compressed by the albumin in the capsular spaces. The capillaries in the interstitial tissue were dilated and filled with red blood cells, although similar cells were rarely found in the tubules. In all the dogs the lesions in the kidneys were diffuse and bilateral. There were no lesions of interest in the genito-urinary tract except those in the kidney.

There were no lesions in the pancreas or in the salivary glands in the one dog in which these glands were examined.

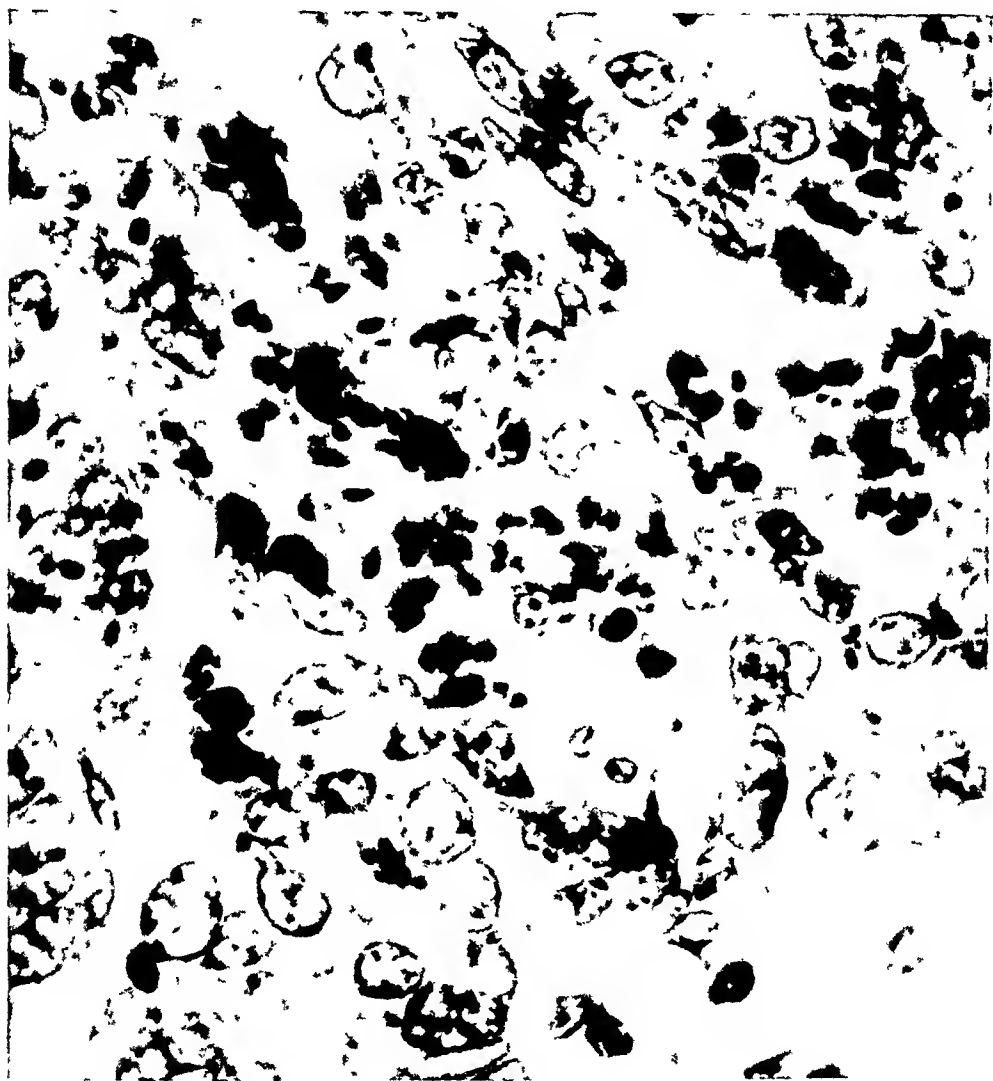


Fig 2—Photomicrograph of the basal portion of the mucosa of the ileum of a dog that received 10 cc of staphylococcus toxin and died six and three-quarters hours later. Note the extensive degeneration of the epithelial cells lining the glands and the accumulation of these cells in the lumens. Similar changes were present throughout the mucosa of the gastro-intestinal tract.

The mesenteric and retroperitoneal lymph nodes were swollen and reddish gray, the sinuses were dilated and filled with red blood cells and phagocytic cells in the dogs in which a large quantity of blood was found in the abdominal cavity.

The only lesions found in the brain were small hemorrhagic areas which were most frequently located about blood vessels.

LESIONS IN THE RABBIT

There were no lesions of interest in the serous cavities

Petechiae were present in the myocardium, endocardium or epicardium, often in all three locations. Few areas of Zenker degeneration were present in the cardiac muscle.

Small hemorrhagic areas were located in the pleura and in the parenchymatous tissue of the lungs. The capillaries in the interstitial tissue were dilated and filled with red blood cells, similar cells were found in groups of the alveoli and in some of the bronchi.

In some of the rabbits the spleen was purplish brown and moderately swollen. The sinuses were filled with red blood cells, polymorphonuclear leukocytes and phagocytic mononuclear cells. Hemosiderin was free in the sinuses and in phagocytic cells.

The liver was purplish brown, when it was sectioned a large amount of blood escaped. In some rabbits small hemorrhagic areas were found in the liver, these had no definite relation to the hepatic lobules or to the blood vessels. The hepatic



Fig. 3—Diffuse necrotic and hemorrhagic foci in the cortex of the kidney of a rabbit that received 0.5 cc of staphylococcus toxin intravenously and twenty-two hours later received an additional dose of 0.75 cc of toxin. The animal died twenty-three and a half hours after receiving the second injection.

cells were compressed about the areas of hemorrhage. A few foci of necrosis were found which were not associated with any evidence of hemorrhage. In some instances polymorphonuclear leukocytes surrounded the necrotic areas.

In one rabbit there was a localized area of hemorrhage and necrosis from 1 to 2 cm in diameter in the mucosa of the cardiac portion of the stomach. The only lesions in the small intestine were a few petechiae in Peyer's patches. The most interesting lesion in the gastro-intestinal tract was found in the first 40 cm of the colon. The wall was often hemorrhagic and edematous, and the mucosa was hemorrhagic or covered with a diphtheritic membrane. In the rabbits in which the hemorrhagic areas were less diffuse the epithelial cells lining the glands showed varying degrees of degeneration. Many of the epithelial cells were completely destroyed, and the fragments filled the lumens of the glands. A few polymorphonuclear leukocytes and mononuclear wandering cells were present in the mucosa of the colon. Circumscribed pink areas, which suggested agglutinated red blood cells or thrombi, filled a few of the capillaries in the mucosa.

The quantity of marrow in the femur varied in the different rabbits. In some rabbits the marrow appeared to be hyperplastic. No lesions were found in either the myeloblastic or the erythroblastic tissue.

There were no lesions in the pancreas or adrenal glands.

The lesions most constantly observed in this series of rabbits were in the kidney. The renal lesions were characterized by cortical necroses and evidence of hemorrhages (fig 3). The necrosis was either focal or diffuse and was always limited to the cortex. The kidneys were swollen and hyperemic in the rabbits that died from two to ten hours after receiving the staphylococcus toxin. The capillaries in the glomerular tufts were dilated and filled with red blood cells, and the tufts almost completely filled the glomerular space. Albumin was sometimes present in the capsular space and in the tubules. In many rabbits the tubular epithelial cells were often swollen and contained hyaline droplets, this was observed especially in the animals that lived for twelve hours or longer after receiving the toxin. Only a few red blood cells were present in the capsular spaces and in the collecting tubules of the kidney. There was a layer of polymorphonuclear leukocytes and phagocytic cells at the margin of the necrotic zone.

COMMENT

The existence of such fractions as nephrotoxin, leukocidin, hemolysin and skin-necrotizing and acute killing fractions in staphylococcus toxin is not discussed here.²²

The potency of staphylococcus toxin is influenced by the organism, mediums and method of preparation.²³ The dog and rabbit, two of the species of animals frequently used in experimental work, have been used in studying the action of staphylococcus toxin. Death occurs in both when a sufficient quantity of a potent toxin is given. The lesions are influenced by the quantity of toxin, the methods of administration and the length of time elapsing before death. The lesions may conveniently be divided into two groups, namely, hemorrhagic and necrotic, in this study the former were more frequently encountered in the dog and the latter, in the rabbit.

Swollen and necrotic endothelial cells are found in the capillaries of the rabbits which survive for twenty-four hours or longer. Collections of red blood cells are present about the periphery of many of the blood vessels, this accounts for the small hemorrhagic areas seen in the viscera of the dogs and rabbits and also for the blood observed in the abdominal cavity of the dogs.

The hemorrhagic and necrotic areas suggest that the toxin either injures the endothelium of the capillaries, causing thrombosis and infarction, or injures the epithelial cells by acting directly on those cells. Although there are thrombi in some of the capillaries in the mucosa of the intestinal tract, there is apparently no direct anatomic relation

22 Weld, Julia T., Parker, and Gunther, Anne. *J. Exper. Med.* **54** 315, 1931.
Burnet.¹²

23 Burnet, F. M. *J. Path. & Bact.* **33** 1, 1930. Parker.¹¹

between the thrombi and the areas of necrosis. Furthermore, the number of thrombi is too small to produce the extensive necrosis found in some of the animals. The thrombi resemble agglutinated red blood cells more than the thrombi formed by leukocytes and fibrin. The presence of areas of hemorrhage and necrosis in the mucosa of the stomach and of the small and large intestine suggests that the toxin is excreted into any part of the gastro-intestinal tract and that the lesions are produced at the point of excretion rather than at the point of accumulation of the toxin. One of the first changes in the intestinal tract is swelling and degeneration of the epithelial cells lining the glands in the deeper portion of the mucosa. If the necrosis of the mucosa is the result of the action of the toxin accumulated in the colon, the superficial portion of the mucosa should show the first and most extensive necrosis.

The intestinal mucosa is one of the routes of excretion of toxic materials. For instance, aluminum when given intravenously is excreted by the mucosa of the stomach and of the small and large intestines.²⁴ Likewise, mercurochrome is quickly excreted into the gastro-intestinal tract of the dog after intravenous administration, as shown by the emesis of intensely red material,²⁵ also, necrosis in the colon of rats following the intravenous injection of mercurochrome has been described by Baldwin.²⁶

The intestinal lesions in the dogs and rabbits after intravenous injection of staphylococcus toxin are similar to those found in guinea-pigs after inhalation of mercurial preparations. In a study of the tissues of guinea-pigs which had been given inhalations of mercurial preparations Gutman²⁷ found evidence of necrosis in the epithelial cells of the villi and of ulceration and shredding of the mucosa in the gastro-intestinal tract. The blood vessels were congested and distended with agglutinated red blood cells, and extravasations of blood were frequently observed. The changes in the large intestine resembled those in the small, however, the ulcerative process was greater in the latter.

While the lesions in the kidney at the time of death differ in the dog and in the rabbit, similar changes are present at times in both species of animals. The absence of cortical necrosis in the dog's kidney can be explained on the basis that death intervenes before sufficient time elapses for the necrosis to occur. Neisser and Wechsberg⁷ con-

²⁴ Underhill, F. P., Peterman, F. I., and Steel, S. L. *Am J Physiol* **90** 52, 1929.

²⁵ Bargaen, J. A., Osterberg, A. E., and Mann, F. C. *Am J Physiol* **89** 640, 1929.

²⁶ Baldwin, W. M. *Proc Soc Exper Biol & Med* **25** 679, 1927.

²⁷ Gutman, J. *Am J Syph* **7** 1, 1923.

sidered the cortical necrosis to be the result of thrombi which were formed by the disintegration of leukocytes. Rigdon and his associates¹⁹ were unable to demonstrate a sufficient number of thrombi to consider the necrosis secondary to obstruction and expressed the opinion that the lesions in the kidney were the result of the action of the toxin on the renal epithelial cells and on the endothelial cells of the capillaries. This explanation apparently is the more plausible one.

It is not surprising that debris, phagocytes and hemosiderin are found in the spleen, for Neisser and Wechsberg⁷ have shown that red blood cells are hemolyzed by staphylococcus toxin, and Van de Velde² has shown that leukocytes are destroyed by it. The presence of a large amount of hemosiderin in the spleen of rabbits dying five minutes after receiving a lethal dose of the toxin suggests that some hemosiderin is present in the spleen of normal rabbits.

In considering the pathogenesis of the lesions in the dog and rabbit two types of lesions must be thought of, namely hemorrhagic and necrotic. The distribution of the former appears to indicate an involvement of the circulatory apparatus, while the latter are usually found in the tissues which have an excretory function. Although there is some evidence in favor of the idea that the necrosis is the result of vascular obstruction, that evidence is not at all conclusive. The two types of lesions may be adequately explained on the basis of direct injury to any cell with which the toxin comes in contact. The endothelial cells of the capillaries are injured, and as a result the permeability of the wall is increased, permitting the escape of red blood cells.

SUMMARY

Hemorrhage and necrosis are the characteristic lesions found in the dog and rabbit after the intravenous administration of staphylococcus toxin. The type of lesion is influenced by the quantity of toxin and the length of time elapsing between the time of injection and the time of death. The areas of necrosis are most frequently found in the kidneys and in the colon of the rabbit while the hemorrhagic areas usually occur in the gastro-intestinal tract and peritoneal cavity of the dog. It appears that the hemorrhages are the result of the action of the toxin directly on the endothelium of the capillaries and small blood vessels, permitting the escape of red blood cells, and that the necrosis is the result of the action of the toxin directly on the cells of the body.

HISTAMINE AND LEUKOCYTOSIS

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MARSHALL M LIEBER, MD

AND

P J KENNEDY, MD

PHILADELPHIA

The presence of histamine in various mammalian tissues has led to intensive investigations to determine its physiologic significance. Some of these¹ indicate that one of its functions has to do with initiating the vascular phases of inflammation following injury to tissue. Pertinent to this is the question what relationship, if any, histamine may have to the leukocytic phenomena associated with inflammation. Experiments have failed to demonstrate that histamine phosphate has any chemotactic effect on leukocytes. The evidence concerning the influence of histamine on the leukocytes in the blood is inconclusive. Some have noted leukopenia, and some leukocytosis, following injections of histamine. Several of the reports are based on incidental observations in which the evidence is fragmentary. There is closer agreement between the reports in which the effect of histamine on leukocytosis was the chief object of the experiment.

Port and Brunow² made an incidental observation on leukocytosis following injections of histamine into a dog. The dog weighed 4.4 Kg and received three injections of 2.2 mg, 3.3 mg and 1.1 mg, respectively, at intervals of several days. Leukocytosis followed each injection and was most marked following the last injection. The authors believed that the previous injections had made the animal more sensitive to the effects of histamine.

Dale and Laidlaw³ reported marked leukopenia accompanying shock due to histamine in cats. The observations were made incidental to studies on the nature and mechanism of shock and on the accompanying changes in blood volume and blood concentration. The leukocytic phenomena were not primary objects of the experiments, and no data showing the number or the intervals of time at which the counts were made

From the Department of Pathology, Jefferson Medical College. Aided by the Martin Research Fund.

1 (a) Lewis, Thomas. *The Blood Vessels of the Human Skin and Their Responses*, London, Shaw & Sons, 1927. (b) Krogh, A. *The Anatomy and Physiology of the Capillaries*, New Haven, Yale University Press, 1929.

2 Port, F., and Brunow. *Arch f exper Path u Pharmacol* **76** 239, 1914.

3 Dale, H. H., and Laidlaw, P. P. *J Physiol* **52** 355, 1919.

were recorded. Cats, under ether anesthesia, received histamine in amounts sufficient to produce death or profound shock promptly. The recorded intervals between the injections of histamine and death ranged from fourteen to thirty-four minutes. The observations must have been made during this period and were obviously intended by the authors to apply only to animals in profound shock immediately following large doses of histamine. The assumption that leukopenia would result at longer intervals following smaller doses of histamine is not warranted by these observations.

Flatow and Huttel⁴ observed the effects of subcutaneous injection of histamine in cats, guinea-pigs, rabbits and dogs. Except in rabbits, this was followed by marked leukocytosis in each case. The blood picture following subcutaneous doses sufficient to produce shock resembled that of severe acute infection. On subsequent days there was an increase in immature forms—"a shift to the left"—followed by an increased number of monocytes and finally by eosinophilia. A transient leukopenia followed shock doses of histamine in rabbits. Paul⁵ found no significant changes in the leukocytic count in rabbits following injections of histamine.

Most of the observations made on human subjects were incidental to the use of histamine for therapeutic purposes, and in most instances the data are fragmentary. Beeri and Weinberg⁶ and Morreti⁷ reported leukopenia following histamine. Motta⁸ injected 1 mg of histamine into the gluteal muscles of five pregnant women. Leukocytic counts were made five, fifteen and thirty minutes following each injection. The results varied. Some counts were slightly higher and some slightly lower than normal, and some showed no significant variation. No subsequent counts were recorded. Andreoli and Lucchi⁹ recorded a moderate leukocytosis in a small group of cases following the therapeutic use of histamine. Weiss, Robb and Ellis¹⁰ found leukocytosis, the degree of which was not stated, following slow intravenous instillation of histamine for therapeutic purposes in five cases.

We have failed to find other observations on a relationship between histamine and leukocytosis either in animals or in man. Since the available evidence is inconclusive, experiments were undertaken to secure additional data. The leukocytes in the blood of adult cats were counted before and at intervals following the intravenous injection of small

4 Quoted by Feldberg, W., and Schilf, E. *Histamin, seine Pharmakologie und Bedeutung für die Humoralphysiologie*, Berlin, Julius Springer, 1930.

5 Paul, J. R. *Bull. Johns Hopkins Hosp.* **32**: 20, 1921.

6 Motta, G. *Arch. d. ostet. e gynec.* **16**: 66, 1929.

7 Andreoli, G., and Lucchi, G. *Minerva med.* **7**: 1117, 1927.

8 Weiss, S., Robb, G. P., and Ellis, L. B. *Arch. Int. Med.* **49**: 360, 1932.

amounts of a sterile solution of histamine phosphate. No anesthesia was used, hence the results may be regarded as uncomplicated by the action of drugs incidental to experimentation. The doses of histamine and the leukocytic counts are presented in table 1.

Differential counts showed that the increase consisted of polymorphonuclear neutrophils. In each instance the blood picture returned to normal within twenty-four hours. In three instances (1, 2 and 4) a decrease in the number of leukocytes was seen within one hour. If subsequent counts had not been made, these results would have indicated that leukopenia follows injections of histamine. But in each instance the leukopenia was transient and was followed by definite leukocytosis.

TABLE 1—*Leukocytic Counts Following Intravenous Injections of Histamine Into Cats*

Experiment	Count Before	Histamine, Mg	Counts After Given Intervals					
			½ Hour	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
1	12,600	2.0	11,800	9,400	16,100	23,700		19,250
2	12,750	1.0	8,800		25,600	22,400	20,350	
3	13,850	2.0		26,400	52,650	38,200	34,900	
4	13,900	2.0		6,200	28,900	32,800	19,000	
5	13,500	1.0		13,700		14,050		24,250
6	10,350	1.0		13,400	25,500			
7	15,200	2.0		17,800	23,800	24,100		
8	12,400	1.5		31,150	34,750	22,000		
9	11,300	2.0	13,800	17,900	24,600	36,000	28,900	
10	13,500	1.5	14,350	16,800	17,350	17,800	33,700	34,300
11	16,300	2.0	20,500	24,500		36,600		28,500
12	13,000	1.5		18,700		21,500		29,100
13	14,200	2.0		20,000				20,800
14	15,600	2.0		21,000		22,250		20,600
15	9,200	2.0	10,700	10,250		24,450		39,900
16	16,000	2.0	20,500	25,300	32,600		20,600	
Average	13,350		14,350	18,160	27,380	25,900	26,240	28,340

Injections of 2 mg. usually produced transient shocklike phenomena. Within one or two hours the behavior and appearance of these cats were not distinguishable from those of cats that had not been treated. Eight cats were used in this experiment, and each one was given injections of corresponding volumes of physiologic solution of sodium chloride for control observations. In no instance was there a significant variation in the leukocyte count following injections of saline solution. In our hands the intravenous injection of histamine produced leukocytosis regularly in cats.

In another experiment monkeys (*Macacus rhesus*) were given subcutaneous injections of varying doses of histamine phosphate in physiologic solution of sodium chloride. Leukocyte counts were made immediately before and at intervals following the injections. Aseptic technic was maintained. Three monkeys were used, and no subsequent

injection was made until the leukocytosis following the previous injection had subsided. Usually three or four days intervened between injections. The pulse and respirations at frequent intervals following the injection of histamine showed slight variations from normal. The manifestations of circulatory disturbances resembling shock were not marked. The doses of histamine and the leukocyte counts are given in table 2.

It will be seen that a sharp rise in the leukocyte count followed the injection of even 0.75 mg. of histamine. The increase consisted of polymorphonuclear neutrophils. The maximum leukocytosis usually occurred from two to four hours following the injection. However, in one instance, when 9 mg. of histamine phosphate was injected, the maximum leukocytosis, 57,550, occurred within an hour. This result may be compared to the high leukocytosis which follows extensive burns of

TABLE 2—*Leukocytic Counts Following Subcutaneous Injection of Histamine Into Monkeys*

Count Before	Histamine, Mg.	Counts After Given Intervals					
		1 Hour	2 Hours	4 Hours	6 Hours	24 Hours	48 Hours
5,550	9.0	57,550	36,800	26,700	20,750	18,950	11,100
6,650	6.0	14,400	16,200	41,400	36,000	17,000	17,150
5,450	4.5	24,150	19,400	7,850	7,780	4,400	9,100
12,050	2.5	25,750	26,100	23,100	24,700	12,300	7,150
7,700	2.25	9,050	22,850	15,000	16,300		12,350
5,480	1.5	20,000	40,000	19,400	18,600	14,300	11,000
6,500	1.0	6,150	18,650	16,650	15,700	11,200	8,000
5,400	0.75	15,400	18,850	18,940	19,650	9,800	9,200

the skin in man. In our hands the subcutaneous injection of histamine was followed by leukocytosis in monkeys.

Histamine phosphate in doses of from 0.5 to 1 mg. in sterile solution was given intravenously to seven young men. The leukocytes in the blood were counted immediately before and at intervals following the injections. A moderate leukocytosis occurred regularly, with an average increase of 3,000 leukocytes from three to five hours following the injection. The count returned to normal within twenty-four hours. In several instances a slight leukopenia was found one hour after the injection. This was followed by an increase in the leukocyte count in each case. Control counts at corresponding intervals were made on the same subjects. In no instance did these show a significant variation. Data concerning this group are shown in table 3.

The intravenous injection of histamine produced a characteristic circulatory reaction. The face at first was flushed but immediately became pale. This was accompanied by a metallic taste in the mouth, dizziness, faintness and frontal headache. The radial pulse was rapid

and sometimes weak. The headache sometimes lasted several hours, but the other manifestations disappeared within thirty minutes. In one instance, case 3, the subject fainted when only 0.5 mg. had been injected. One hour later his leukocytic count had decreased from 5,900 to 1,700. The count rose immediately to 9,800, and twenty-four hours later it was 9,000. This was the only instance in which the leukocytosis in our subjects persisted twenty-four hours.

TABLE 3—*Leukocytic Counts Following Intravenous Injection of Histamine into Human Subjects*

Subject	Normal Count	Histamine, Mg	Counts After Given Intervals		
			1 Hour	3 Hours	5 Hours
1	5,900	0.75	9,050	9,700	8,750
2	7,900	0.75	8,350	9,000	9,150
3	5,900	0.50	1,700	9,800	8,750
4	6,350	1.00	6,250	9,450	6,600
5	8,900	0.75	7,650	8,400	15,000
6	6,500	1.0	5,800	4,350	8,200
7	7,800	1.0	5,350	13,600	14,100

TABLE 4—*Leukocytic Counts Following Subcutaneous Injection of Histamine Into Human Subjects*

Subject	Count Before	Histamine, Mg	Counts After Given Intervals				
			1 Hour	3 Hours	5 Hours	7 Hours	24 Hours
1	5,700	2.0	7,650	6,600	7,700		
2	6,240	2.0	6,000	7,150	5,300		6,200
3	7,100	2.0	7,000	6,500	7,200		7,150
4	4,800	2.5	5,650	9,450	6,000		5,000
5	6,500	3.0	6,950	7,200	8,700		6,050
6	6,750	3.0	5,250	5,000	7,200		5,650
7	5,150	5.0	3,800	6,800	7,600	7,600	5,000
8	8,500	5.0	6,500	10,450	11,300	10,600	8,600
9	5,350	5.0	4,400	8,600	8,650	9,600	5,100
10	6,400	5.0	5,300	9,700	10,150	8,700	6,200

Histamine phosphate was given by subcutaneous injection to another group of volunteers. In these the results varied with the amounts of histamine given. In three subjects receiving 2 mg. the leukocyte counts remained within normal limits throughout the course of the experiment. Subject 4, receiving 2.5 mg., showed a maximum rise of 4,650 leukocytes in three hours. Subject 5, receiving 3 mg., showed a maximum increase of 2,200 in five hours, but subject 6, receiving the same dosage, showed little or no effect. In the four subjects given 5 mg. of histamine phosphate a decrease in the number of leukocytes occurred in the first hour followed regularly by an average increase of 3,340 leuko-

cytes in from five to seven hours. Control counts made at corresponding intervals on another day showed no significant variations from normal. The polymorphonuclear neutrophils were the only cells showing significant numerical variations both in this group and in those receiving histamine intravenously.

The subcutaneous injections produced general manifestations similar to those following intravenous injections, but less in degree. Rapid pulse and flushing of the face were noted within a few minutes. A few of the men had headache, which lasted several hours, some noticed shortness of breath on exertion, in others these effects were not present. The local effects were less marked than had been expected. The injections were made into the loose areolar tissue near the elbow. They resulted in slight local swelling and tenderness to pressure for from twenty-four to forty-eight hours. In no case did this cause complaint, and it was regarded as trivial by each of the subjects.

COMMENT

It has been shown (Lewis^{1a}) that any type of injury to the human skin results in a circulatory reaction which is local in origin and is independent of innervation. This reaction depends on the release of a diffusible substance from the injured cells, which substance is responsible for initiating the early phases of inflammation about the point of injury. Lewis could not distinguish this substance from histamine but, lacking evidence concerning its exact chemical nature or combination, he speaks of it as H-substance. Dale⁹ confirmed the conclusions of Lewis, and stated that there is as good chemical evidence of the presence of histamine in the cells of the body generally, and of its liberation from them following injury, as of the existence of epinephrine in the adrenal medulla, and of its secretion as such into the blood.

Lewis attributed the systemic phenomena which follow extensive superficial injuries, such as burns, to the liberation of large amounts of H-substance from the injured skin. It is well established that shock, similar in all respects to that resulting from injection of histamine, follows extensive superficial burns. It also is well known that such burns are followed immediately by marked leukocytosis. Locke¹⁰ reported counts ranging from 10,000 to 50,000 within two and one-half hours following burns. The leukocytic counts were above 50,000 regularly in the fatal cases. This raises the question whether such leukocytosis is due to H-substance or to some other agent, and whether H-substance is identical with histamine in its influence on leukocytes.

9 Dale, H. H. *Lancet* **1** 1235 1929

10 Locke, E. A. *Boston M. & S. J.* **147** 480, 1902

Physiologic assays (Lewis^{1a}) indicate that histamine, estimated in terms of the base, is present in the human skin in a concentration of about 1/60,000. A simple calculation shows that 5 mg of histamine phosphate, which contains approximately 2.5 mg of the base, will be contained in about 150 Gm of skin. In several of our human subjects the subcutaneous injection of 5 mg of histamine phosphate was followed by a leukocytosis of 10,000. This approximates relatively, though perhaps it does not equal, the leukocytosis which follows moderately extensive burns of the skin. However, the experimental leukocytosis is very similar in degree to that which regularly follows extensive surgical procedures when no infection is present. Postoperative leukocytosis may be due in part to the absorption of histamine from areas of traumatized tissue.

It must be remembered that the chemical combination in which histamine exists in tissues is not known. It is possible that that combination is more effective in its physiologic action than is histamine phosphate.

Higher leukocytic counts were obtained in cats and in monkeys than in man. This probably was due to the larger dose relative to the body weight.

We express our appreciation to the group of medical students who voluntarily submitted to experimentation in the work here reported.

CONCLUSIONS

Histamine phosphate given intravenously to cats, subcutaneously to monkeys and by both methods to man is followed by an increase in the number of polymorphonuclear leukocytes in the blood.

Frequently the leukocytosis following injections of histamine is preceded by a transient leukopenia.

The release of histamine from cells in areas of extensive injury is probably a factor in evoking the subsequent leukocytosis. This may account for the leukocytosis following surgical and other injuries.

BEHAVIOR OF TRANSPLANTED SPLEEN

WITH SPECIAL REFERENCE TO THE TISSUE DIFFERENTIAL OF
HEMOPOIETIC ORGANS

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Various hemopoietic organs can be transplanted, but with much less success than epithelial tissues. I wished to analyze this deficiency in transplantability, and for this purpose I studied transplantation of splenic tissue in guinea-pigs. In particular it seemed of interest to determine, if possible, (1) how regeneration of splenic tissue takes place under the conditions of transplantation, and (2) why hemopoietic tissues show only very slight regenerative and growth tendency.

MATERIAL AND METHODS

Autotransplantations, syngenesiotransplantations and homeotransplantations of splenic tissue were carried out in guinea-pigs weighing from 200 to 250 Gm. Pieces of spleen, about 4 by 4 mm, were transplanted immediately after removal into subcutaneous pockets of the abdominal wall. The grafts were taken out together with the surrounding tissue and cut in serial sections. Twenty-four animals were used in these experiments.

OBSERVATIONS

Autotransplantation—After two days the peripheral zone of the graft is alive, the reticulum cells are producing free wandering phagocytotic histiocytes. Living malpighian bodies are also seen, but show no signs of regeneration, a slight invasion of the transplant by some lymphocytes from the blood stream of the host is noted. The central zone of the transplant has become necrotic in some places. There are here no invading leukocytes and lymphocytes, no mobilization of reticulum and endothelial cells is noticeable, nor is there any marked activity of the malpighian bodies. The power of resistance of the latter is striking. A certain number of the lymphocytes in these structures are alive, their cytoplasm somewhat increased, but other lymphocytes are damaged or destroyed. An augmentation of mitotic figures is not observed. Four days after transplantation the inflammatory cells in the peripheral zone have disappeared. Here the reticulum cells have become mobilized and transformed into histiocytes, which are numerous and are active phagocytes. Many lymphocytes of the

malpighian bodies show an increase in cytoplasm, and some of these cells also have become free wandering cells, having thus been changed into polyblasts. The central zone of the graft shows signs of injury, some lymphocytes being destroyed. However, here also a large number of lymphocytes of the malpighian bodies are well preserved, and they likewise become converted into polyblasts which penetrate through the necrotic zone and reach the living tissue in the periphery. The polyblasts thus have two points of origin. Some arise from the peripheral living zone of the lymphoid tissue as well as from the transformed reticulum cells in this region, while others develop from the surviving cells in the central zone. Mitotic figures are seen everywhere in the lymphocytes, but their number is not increased over the normal. In the interior of the graft neither the fibrocytes nor the undifferentiated mesenchymal cells are augmented. After eight days the transplant is surrounded by loose vascularized fibrous tissue. The regeneration within the graft itself is progressing and takes place in the reticulum and the lymphoid tissue of the peripheral zone as well as in the central zone. In some areas there is an outgrowth of endothelial cells of the sinuses toward the center of the piece, producing small spaces filled with blood and lymph cells. Many newly formed small and medium-sized lymphocytes are found, while the previously necrotic central zone is replaced by a great number of phagocytic polyblasts. Associated with the new formation of cells there is a new production of loose reticulum fibers. This process is still more evident after twelve days. After twenty-one days the transplanted tissue on the whole is apparently well preserved, displaying again the normal structure of spleen. The endothelial cells of the sinuses show no mobilization, but they still continue to grow out in some places. The undifferentiated mesenchymal cells and hemocytoblasts are not increased, but are present in their usual number. In the periphery of the transplant a conglomeration of eosinophilic leukocytes is found. The graft itself is surrounded by a large dense well vascularized layer of fibrocytes forming a connective tissue capsule.

Syngeneisotransplantation—After two days the periphery as well as the center of the transplant has become necrotic to a considerable extent. Trabeculae are still evident, and sinuses and malpighian bodies are invaded for the most part by lymphocytes. In the center remnants of malpighian bodies are found with greatly injured lymphocytes. The living reticulum cells are transformed into free wandering cells, but no transformation into lymphocytes is observed. The small and medium-sized lymphocytes, so far as they are undamaged, have produced polyblasts of various shapes. The reticulum fibers have become swollen, they are spread out and partly dissolved. After four days the resorption processes in the graft have progressed still farther. The reticulum

fibers are for the most part destroyed, the tissue itself is invaded by lymphocytes and a great many polyblasts coming from the blood stream of the host. The lymphoid cells of the transplant have become necrotic, the malpighian bodies damaged. Some of the reticulum cells are mobilized and transformed into phagocytic histiocytes invading the periphery. After eight days a considerable production of fibrocytes is seen surrounding the transplant, while histiocytes are found all over the graft. These occur chiefly at the periphery, and their number is diminished toward the central part. This finding, in addition to the fact that in earlier stages the histogenous polyblasts produced inside the transplant have become mostly necrotic, suggests that the majority of the histiocytes are of hematogenous origin from the host. Accordingly, after from twelve to twenty-one days, the graft is almost completely replaced by dense masses of connective tissue showing hard collagenous and softer reticulum fibers. Only very few remnants of trabeculae and sinuses with well preserved endothelium are noticeable. The proliferation of fibrocytes indicates that formative processes on the part of the antagonistic mesenchyme of the host lead to an overwhelming of the transplant by host elements.

Homeotransplantation—As in the syngenesiotransplant, one notices here, also, as early as after two days numerous necrotic areas in the center of the homeotransplant. The reticulum fibers have obviously become dissolved, while the lymphocytes have been mostly destroyed. A certain resistance of the endothelial cells is found. The invasion of the graft by lymphocytes is pronounced. Masses of hematogenous polyblasts from the host are actively wandering toward the center, but no evidence of any considerable reaction of the lymphoid, reticulum or endothelial cells of the graft can be detected. Necrotic remnants of malpighian bodies are still present, whereas in the periphery some trabeculae and sinuses are sometimes alive. Here and there a histogenous reaction on the part of the graft is noted. But as a rule all over the transplant it is the polyblasts coming from the vessels of the host which prevail, and they decrease in number toward the necrotic central zone. After eight days scanty remnants of the spleen are still evident in the periphery, the center has become completely necrotic. A great number of histiocytes are present showing phagocytosis. A layer of well vascularized connective tissue has surrounded the graft. After twelve days the transplant has been for the most part replaced by polyblastic histiocytes and fibrocytes with a formation of fibers growing toward the periphery. A striking ingrowth of capillaries is seen as well as a considerable migration of eosinophilic leukocytes into the graft. After twenty-one days these leukocytes are no longer visible. A perfect organization has taken place. However, in the periphery of the trans-

plant trabeculae and the last remnants of sinuses may still be recognized. A well vascularized connective tissue replaces the transplant.

COMMENT AND CONCLUSIONS

Autotransplants manifest a certain growth and full regenerative tendency, which is evident after twenty-one days. All the elements of the spleen are able to regenerate.

1 Endothelial cells show typical outgrowth in a lengthwise direction, forming new venous and lymph sinuses.

2 Reticulum cells have a marked power of resistance to injury connected with transplantation and produce phagocytes, but there is no evidence of their transformation into lymph cells.

3 Lymphocytes, so far as they remain alive, show two kinds of reaction:

(a) Some increase in number as a result of mitotic proliferation which leads to a new formation of small and medium-sized lymphocytes. This takes place especially in the malpighian bodies.

(b) Others undergo hyperplastic changes leading to the production of polyblasts. Later on these become fixed, new fibers being produced.

4 Hemocytoblasts and indifferent mesenchymal cells exert their power of further differentiation.

The graft itself is surrounded by a massive fibrous capsule, which, in general, is not usual in autotransplants.

In syngenesiotransplants and homeotransplants full regeneration was not noted. As to the origin of the phagocytes one has to consider as possible sources: (1) reticulum cells of the graft, (2) surviving lymphocytes of the graft, (3) nongranular white cells of the host, (4) preexisting histiocytes of the connective tissue of the host.

Without doubt, some of the histiocytes are formed from the transplanted reticulum cells. As to their derivation from preexisting lymphocytes, I¹¹ have demonstrated that such a transformation certainly occurs. Many of these phagocytes in the earlier stages following transplantation were seen in the center proceeding toward the periphery, where they were destroyed. In later periods a new accumulation of these phagocytic cells was observed in the periphery, together with an invasion by leukocytes, at a time when the common lymphocytes had disappeared. Such observations indicate that a transformation of the invading lymphocytes and monocytes into histiocytes must have taken place. The number of preexisting monocytes in blood and lymph is too small to explain

1 Silberberg, M. (a) *Virchows Arch f path Anat* **274** 820, 1930, (b) in Hirschfeld, Hans, and Hittmair, Anton. *Handbuch der allgemeinen Hamatologie*, Berlin, Urban & Schwarzenberg, 1932, vol 1, p 2.

completely the considerable number of polyblasts which are found in these grafts. Subsequently the graft is absorbed by these phagocytes, which at a still later time are transformed into fibrocytes. The pre-existing histiocytes of the host's connective tissue likewise are too few to explain the formative processes which take place within the graft.

According to Jaffe and Richter,² regeneration of lymphocytes in transplants of splenic tissue is effected mostly by hyperplastic reticulum cells. They did not, however, distinguish between the indifferent mesenchymal cells and the reticulum cells. Though a number of more recent experiments³ have clearly shown that a transformation of lymphocytes and reticulum cells into polyblastic histiocytes is possible, a transformation of histiocytes into lymphocytes is not proved. While a development of lymph cells from the indifferent mesenchymal cells directly or by way of hemocytoblasts is quite possible, such an origin would be exceptional.

Maine and Manley⁴ have pointed out that in autotransplants of spleen survival and growth are the rule. I noted a variable growth tendency. I used young guinea-pigs, which may account for the fact that I had better results with homeotransplants than the last named investigators. I could not find any evidence for their statement that removal of the spleen acts as a powerful stimulus to the growth of the graft. Considering the work done in tissue cultures one knows that the sharper the cut edges of a piece are the better is its growth in culture. It may be that grafts with many sharp cuts would show better growth than those with only a few cuts.

All the experiments made so far make it evident that tissue from hemopoietic organs has no marked resistance to transplantation (Loeb⁵), while there is no doubt that epithelial organs and their cells, as a rule, show better regenerative power. Ordinary connective tissue also has a high growth tendency. It may therefore be assumed that the tissue differential^{5d} of epithelial and mature connective tissues is firm and constant, while that of the hemopoietic tissues, in spite of their natural variability, can be less readily maintained.

Whenever hemopoietic tissues are placed in certain mediums or even merely removed from their original position within the body one finds a definite result. In tissue cultures as well as in syngenesiotransplants

2 Jaffe, H. L., and Richter, M. N. *J. Exper. Med.* **47** 917, 1928.

3 Maximow, A., in von Mollendorff, Wilhelm. *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1927, vol. 2, p. 1. Bloom, W., in Hirschfeld, Hans, and Hittmair, Anton. *Handbuch der allgemeinen Hamatologie*, Berlin, Urban & Schwarzenberg, 1932, vol. 1, p. 20. Silberberg¹.

4 Maine, D., and Manley, O. T. *J. Exper. Med.* **32** 113, 1920.

5 Loeb, Leo. (a) *Arch. Path.* **12** 203, 1931, (b) *American Naturalist* **65** 385, 1931, (c) *Biol. Bull.* **40** 143, 1921, (d) *Physiol. Rev.* **10** 547, 1930.

and homeotransplants of spleen all the various well characterized elements of this tissue undergo changes which finally lead to their transformation into fibrocytes, thus indicating a loss of their characteristic tissue differential

Presumably the changes in the nature of the surrounding fluid medium which take place under these conditions, and perhaps also the lack of connection with the regulating influence of nerves, lead to the loss of the cellular characteristics of these mesenchymal cells. In forming fibrocytes the cells of the mesenchyme undergo the least specific differentiation. At the same time the reactions on the part of the host's mesenchyme prevail over the grafted hemopoietic tissues, the latter seem to elicit a much stronger homeotoxic reaction in the surrounding tissue of the host than do other transplanted tissues. In autotransplants, vascularization taking place and the body fluids remaining the same, the conditions for regeneration and growth are better, these grafts therefore remain alive a longer time, and their cells retain their specific character.

SUMMARY

Autotransplants of spleen manifest positive growth and full regenerative tendency.

The regeneration of this organ is characterized by a specific and remarkably balanced growth of all its different cells, it is usually completed within from sixteen to twenty-one days following transplantation.

In syngenesiotransplants and homeotransplants a full regeneration does not occur.

The cytologic observations reported here strongly suggest a transformation of lymphocytes into polyblasts and histiocytes.

Lymphoid and hemopoietic tissue, in general, have no marked resistance to transplantation.

It may be assumed that the tissue differential of epithelial organs and connective tissues is pronounced, while that of the hemopoietic tissues, in spite of the variability of their cells, is less marked. The results of transplantation and explantation of hemopoietic tissue may be explained by a loss of their tissue differential.

EFFECT OF HYPOPHYSECTOMY ON NATURAL RESISTANCE OF ADULT ALBINO RATS TO HISTAMINE POISONING

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AND

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The removal of the adrenal glands of rats is followed by a marked depression in the natural resistance to various toxins, poisons and bacterial and protozoan infections. The depression is associated to a greater degree with the loss of cortical than with that of medullary substance. This conclusion is based on the fact that the administration of epinephrine to adrenalectomized rats influences the resistance only to a slight degree,¹ whereas the administration of the adrenal cortical hormone may raise the resistance almost to the normal level.²

The work of Smith³ and other investigators demonstrated that removal of the hypophysis in rats is followed by atrophy of the adrenal cortex as well as of the lymphatic tissue, gonads, thyroid gland and thymus. The atrophy of the adrenal cortex is, however, unassociated with changes in the medullary portion of the gland. The experiments reported in the present communication were undertaken to determine the effect of hypophysectomy on the natural resistance of rats to histamine poisoning and to correlate the anatomic changes in the adrenal gland with the changes in resistance. In the course of the work it was possible to determine that the posterior lobe of the hypophysis played no significant part in the maintenance of resistance to this poison.

While this work was in progress, Wyman and tum Suden⁴ reported studies on the reactions of hypophysectomized rats to histamine. They observed that after total hypophysectomy the minimum lethal dose of histamine may or may not be reduced to one half.

From the Laboratory Division, Montefiore Hospital for Chronic Diseases

1 Perla, D, and Marmorston-Gottesman, J. *Am J Physiol* **89** 152, 1929

2 Perla, D, and Marmorston-Gottesman, J. *Proc Soc Exper Biol & Med* **28** 1022, 1931

3 Smith, P E. *Am J Anat* **45** 205, 1930

4 Wyman, L C, and tum Suden, Caroline. *Am J Physiol* **109** 115, 1934

METHOD OF INVESTIGATION

All the rats used in these studies were of an original Wistar Institute stock raised in our laboratory during a period of twelve years⁵. At the time of operation the rats used were between 3 and 4 months of age. The hypophysis was removed, according to the method described by Smith, through the ventral parapharyngeal route. The approach was through the nasopharynx. Precautions were taken in the postoperative care of the animals to maintain a high temperature, as hypophysectomized animals are very sensitive to cold.

Of seventy-six rats that were hypophysectomized completely or partially and that survived the operation, thirty-two on which there was no further intervention died in from two to eighty-eight days⁶. The remaining forty-four rats were given injections of varying amounts of histamine at varying intervals after hypophysectomy. Some of these animals were given repeated injections at long intervals of increasing amounts of histamine in an effort to determine the lethal dose. Autopsies were performed on all the rats, and the percentage weights of the liver, spleen, kidneys, thyroid gland, thymus, adrenals, ovaries, tubes and uterus, testes, seminal vesicles and prostate were determined. Sections of all the organs were studied histologically, and the hypophyseal region was sectioned and examined histologically for fragments. In many instances gross or microscopic fragments of the anterior lobe were noted, but in none were fragments of the posterior lobe observed. Under these circumstances the effect on the resistance of fragments of the anterior lobe could be estimated. The susceptibility of the rats to histamine poisoning was studied.

OBSERVATIONS

It was observed that loss of weight of the animal and atrophy of the testes were not reliable criteria of complete removal of the anterior lobe. In seven instances marked atrophy of the testes was observed in the presence of fragments of the anterior lobe of the hypophysis that were visible grossly. The adrenal glands showed striking changes within nine days after hypophysectomy. Hemorrhage appeared in the reticular zone of the cortex sometimes as early as the third day after hypophysectomy. The presence of cortical hemorrhage was noted in rats that showed no evidence of infection and received no histamine. In other rats large macrophages filled with hemosiderin⁷ were observed between the cortex and the medulla. The hemorrhagic change in the adrenal cortex in adult rats after hypophysectomy⁸ apparently precedes the atrophy of the cortex described by Smith³.

Transient diabetes insipidus, which disappeared within four or five days after the operation, was frequently noted in completely hypophysectomized rats. During this period the increased excretion of water was so marked that the fur of the rat was constantly wet about the genitalia. In partially hypophysectomized rats the increased excretion of water was not prominent, though an increase in the intake of water occasionally occurred. Accurate quantitative determinations were

5 The rats were of Bartonella-carrier stock. Smears of the blood and blood counts were made on a number of the animals in the course of the experiment, but the latent infection was apparently uninfluenced by hypophysectomy.

6 Extreme sensitivity to cold is a striking feature. A high mortality after hypophysectomy occurs if the temperature of the postoperative environment is permitted to drop below 80 F. Sensitivity to cold after this operation is even more striking than after adrenalectomy.

7 The pigment appeared blue with the Turnbull stain for iron pigment.

8 Perla, David. *Proc Soc Exper Biol & Med* **32** 655, 1935.

not, however, consistently recorded. In a recent study Richter⁹ reported transient diabetes insipidus in completely hypophysectomized rats and permanent diabetes insipidus in rats in which the posterior lobe and part of the anterior lobe were removed.

Effect of Hypophysectomy on Resistance of Rats to Histamine Poisoning One Week After Operation—Within the first nine days thirty-three rats on which operation was performed died spontaneously. In eight instances local cellulitis at the site of operation occurred. In one purulent urethritis was present. Sixteen rats died of shock within forty-eight hours. In eight of the twenty-one animals surviving for from three to nine days there were hemorrhagic changes in the adrenal glands. Infection of the sinuses occurred in a few rats.

Twenty-three rats received histamine seven to nine days after operation in amounts varying from 150 to 500 mg per kilogram of body weight. In nine of these animals fragments of the anterior lobe were observed at autopsy, in six the

TABLE 1—*Effect of Hypophysectomy on Resistance of Rats to Histamine Poisoning Seven to Nine Days After Operation*

Operation	Number of Rats	Histamine, Mg per Kg	Number Survived	Number Killed
Complete hypophysectomy	1	150	1	0
	3	200	1	2
	1	300	0	1
	1	400	0	1
Partial hypophysectomy	1	150	1	0
	6	200	6	0
	2	400	1	1*
	1	500	1	0
Exposure of hypophysis without removal	2	400	2	0
	1	600	1	0
	1	900	1	0
Normal controls	2	900	1	1
	2	1,000	0	2

* The adrenal glands were atrophic, the fragment of the anterior lobe of the hypophysis was minute.

hypophysis was completely removed, and in four the hypophysis was exposed but not removed (controls on which operation was performed). Four normal rats received 900 and 1,000 mg of histamine per kilogram of body weight. As may be seen in table 1, the hypophysectomized rats were killed by histamine in amounts as small as 200 mg per kilogram, whereas the controls survived injections of 900 mg per kilogram. The resistance of the hypophysectomized rat one week after operation was about one-fifth that of the normal rat.

Effect of Hypophysectomy on Resistance to Histamine Poisoning from Two to Four Weeks After Operation—In this group fifty-five tests were performed on thirty-eight rats, three of which were normal animals used as controls. In twelve instances the hypophysis was observed to be completely removed. In twenty-five instances fragments of the anterior lobe were noted, and in fifteen the hypophysis had been exposed but was not disturbed (controls on which operation was performed). The interval between the time of operation and that of injection varied from thirteen to twenty-eight days. The amount of histamine administered to the animals which had been operated on varied from 150 to 900 mg per kilogram of body weight. The three normal animals used as controls received 900, 1,000 and 1,100 mg of histamine per kilogram of body weight.

As may be noted in table 2, the completely hypophysectomized rats were killed by histamine in doses as small as 300 mg per kilogram of body weight, while the partially hypophysectomized rats survived doses of as much as 600 mg per kilogram. The controls which were operated on and the normal controls survived the injection of as much as 900 mg per kilogram of body weight. In one partially hypophysectomized rat that was killed by 600 mg of histamine per kilogram, the adrenals were small, and evidence of old hemorrhage in the reticular zone of the cortex was seen. In another rat which was killed by 500 mg per kilogram, the adrenals were also small, although a large fragment of the anterior lobe of the hypophysis was present. In one of two rats used as controls on which operation was performed and which died after receiving 900 mg of histamine per kilogram, autopsy revealed a localized abscess in the neck at the site of operation.

TABLE 2—*Effect of Hypophysectomy on Resistance to Histamine Poisoning Two to Four Weeks After Operation*

Operation	Number of Rats	Histamine, Mg per Kg.	Number Survived	Number Killed
Complete hypophysectomy	3	150	3	0
	2	200	2	0
	5	300	3	2
	1	400	0	1
	1	500	0	1
Partial hypophysectomy	3	150	3	0
	4	200	4	0
	7	300	7	0
	5	400	5	0
	3	500	2	1*
	2	600	1	1†
Exposure of hypophysis without removal	5	200	5	0
	1	300	1	0
	1	400	1	0
	2	500	2	0
	2	600	2	0
	2	800	2	0
	2	900	1	1
Normal controls	1	900	1	0
	1	1,000	0	1
	1	1,100	0	1

* The adrenal glands were small.

† Evidence of old hemorrhage in the cortex (reticular zone) and of recent hemorrhage was noted.

In two of the rats in which fragments of the anterior lobe of the hypophysis remained the adrenal cortex was atrophic, and in three the weight of the gland was only slightly less than normal. The testes were atrophic in three animals, but the seminal vesicles and prostate were definitely atrophic in six. The ovaries and tubes showed regressive changes in two animals. Apparently, then, atrophy of the gonadal tissue may occur in the presence of fragments of the anterior lobe of the hypophysis, and the cortex of the adrenal may also show involutional changes in the presence of such fragments.

From this experiment it appears that the minimum lethal dose of histamine for hypophysectomized rats from two to four weeks after operation is equivalent to about one-third the lethal dose for normal rats (table 2).

Effect of Hypophysectomy on Resistance to Histamine Poisoning from Five to Ten Weeks After Operation—In this group forty-one tests were made on thirty-seven rats. Four of these tests were performed on normal rats used as controls,

eight on rats in which the hypophysis was completely removed, fifteen on rats in which fragments of the anterior lobe were noted at autopsy and fourteen on rats in which the hypophysis was exposed but not disturbed (controls on which operation was performed)

Two of the normal rats used as controls received 1,000 mg and two 1,200 mg of histamine per kilogram of body weight. The rats which had been operated on received histamine in doses ranging from 300 to 1,000 mg per kilogram of body weight.

In table 3 it is shown that the completely hypophysectomized rats were killed by histamine in doses of 600 mg per kilogram of body weight and above but that they survived smaller doses. The partially hypophysectomized rats survived doses

TABLE 3—*Effect of Hypophysectomy on Resistance to Histamine Poisoning Five to Ten Weeks After Operation*

Operation	Number of Rats	Histamine, Mg per kg	Number Survived	Number killed
Complete hypophysectomy	1	300	1	0
	2	400	2	0
	1	600	0	1
	2	800	0	2
	1	900	0	1
	1	1,000	0	1
Partial hypophysectomy	3	400	3	0
	4	600	3	1*
	4	700	0	4†
	2	800	0	2
	2	1,000	0	2
Exposure of hypophysis without removal	1	400	1	0
	1	600	1	0
	2	700	2	0
	2	800	2	0
	6	900	5	1
	2	1,000	1	1
Normal controls	2	1,000	1	1
	2	1,200	0	2

* The adrenal glands showed evidence of old and recent hemorrhages.

† Two of these were pregnant females—one with bronchopneumonia and one with atrophy of the adrenal glands.

as large as 600 mg per kilogram of body weight. The rats used as controls on which operation was performed survived doses of as much as 1,000 mg per kilogram of body weight, the operative procedure having no effect on the resistance to histamine. The normal animals used as controls survived injections of as much as 1,000 mg per kilogram.

The adrenal glands of the completely hypophysectomized rats were found to be atrophic at autopsy, the cortex being smaller than normal.

Of the four partially hypophysectomized rats which were killed by 700 mg of histamine per kilogram, two were pregnant at the time of injection, one had intercurrent bronchopneumonia and a localized abscess in the hypophyseal region and the fourth, though possessing a fragment of the anterior lobe of the hypophysis, had atrophic adrenals. Of the partially hypophysectomized animals in which the adrenals were found to be of normal size, three survived a dose of 600 mg per kilogram, and two were killed by injections of 800 mg per kilogram. The fragments of the anterior lobe of the hypophysis noted at autopsy in this group of rats

were apparently viable and often showed evidence of hypertrophy. The presence of a fragment of the anterior lobe is sufficient to maintain a high degree of resistance to histamine unless the adrenals are atrophic (table 3).

In four of the group of rats on which tests were made from five to ten weeks after operation localized spontaneous infections were observed, all in the vicinity of the operative site. In two of the controls on which operation was performed localized abscesses were noted beneath the hypophyseal membrane, but not invading it. Neither animal died of histamine poisoning. In one of the partially hypophysectomized rats a small abscess was observed in the hypophyseal area, though not destroying the fragment.

It is evident that removal of the pituitary gland in rats from five to ten weeks prior to injection of histamine definitely diminishes the natural resistance of these animals to histamine to about one-half that of normal rats. Even in the presence of a small fragment of the anterior lobe there may be a marked decrease in adrenotropic substance in some instances, as indicated by atrophy of the adrenal cortex.

TABLE 4—*Effect of Partial Hypophysectomy on Resistance to Histamine Poisoning Thirteen to Twenty-Six Weeks After Operation*

Operation	Number of Rats	Histamine per Kg of Body Weight, Mg	Number Survived	Number Killed
Partial hypophysectomy	2	600	1	1
	1	700	1	0
	3	800	2	1
	3	900	1	2
	1	1,000	0	1
Exposure of hypophysis without removal	1	900	0	1
	2	1,000	1	1
	2	1,100	0	2
Normal controls	1	900	1	0
	1	1,000	0	1
	1	1,100	0	1

Since the medulla is apparently unaffected by hypophysectomy, it is probable that the decrease in resistance to histamine after this procedure is associated with the cortical atrophy.

Effect of Partial Hypophysectomy on Resistance to Histamine Poisoning, from Thirteen to Twenty-Six Weeks After Operation—In this group tests were made on eighteen rats. Of these three were normal. In ten rats fragments of the anterior lobe were noted, which in some instances were apparently hypertrophied to from one-third to two-thirds the size of the normal anterior lobe. The posterior lobe was absent in all of these animals. In five rats the hypophysis had been exposed but was undisturbed (controls on which operation was performed). In no instance was the hypophysis completely removed.¹⁰ The partially hypophysectomized rats received from 600 to 1,000 mg of histamine per kilogram of body weight. Five controls on which operation was performed received histamine in doses of from 900 to 1,100 mg per kilogram. In table 4 it is shown that the partially hypophysectomized rats survived doses of as much as 900 mg of histamine per kilogram, though in a few instances they were killed by smaller doses.

¹⁰ Adult rats do not survive complete hypophysectomy as well as immature rats. At the time of this experiment we were not successful in keeping completely hypophysectomized rats alive for longer than three months.

It is striking that all the partially hypophysectomized rats surviving the injections of histamine possessed adrenal glands of normal size and appearance, even though in some instances the gonads or other tissues showed atrophic changes. The rats with atrophy of the adrenal glands were in all instances killed by histamine in amounts less than the minimum lethal dose for normal rats. However, in one instance a rat with apparently normal adrenals was killed by 800 mg per kilogram.

Effect of Adrenal Cortical Hormone on the Natural Resistance of Hypophysectomized Rats to Histamine—Since the decrease in resistance to histamine in hypophysectomized rats was associated with atrophic changes of the adrenal cortex, the effect of repeated injections of adrenal cortical hormone on the natural resistance of hypophysectomized rats to histamine poisoning was studied.

Seventeen totally hypophysectomized adult albino rats were divided into two groups. One group of nine hypophysectomized rats from five to eight days after operation were given intraperitoneal injections twice daily during a period of six days of adrenal cortical hormone¹¹ in amounts of 1 cc daily for each rat (equivalent to 40 Gm of fresh ox. cortex). The other group of eight hypophysectomized rats received no cortical hormone. Six days after treatment was begun the rats treated with cortical hormone were given injections of histamine in amounts ranging from 600 to 800 mg per kilogram of body weight. The untreated hypophysectomized rats received histamine in amounts ranging from 200 to 700 mg per kilogram. Two rats used as controls on which operation was performed received 600 and 900 mg, respectively, of histamine per kilogram of body weight. Of four normal rats used as controls two received 900 mg of histamine per kilogram and two 1,000 mg.

The rats treated with cortical hormone in most instances appeared to be improved clinically and gained in weight, though there was no anatomic effect on the atrophied adrenal cortex.

In table 5 it is shown that of the nine hypophysectomized rats treated with cortical hormone six survived injection of histamine in doses of from 600 to 800 mg per kilogram of body weight and three were killed by doses of 700 and 800 mg per kilogram.

The hypophysectomized rats not treated with cortical hormone and receiving injections of histamine were killed by doses as low as 200 mg per kilogram of body weight and did not in any instance survive a dose of more than 400 mg per kilogram.

The repeated injections of large amounts of adrenal cortical hormone raised the natural resistance of totally hypophysectomized adult rats to histamine poisoning in some instances almost to the level of that of normal rats (table 5). It will be noted that this increase in resistance was effected during the first two weeks after hypophysectomy, when the greatest decrease in natural resistance to histamine was observed to occur. Whereas the minimum lethal dose of histamine for hypophysectomized rats within this period varied from 200 to 300 mg per kilogram of body weight, the hypophysectomized rats treated with cortical hormone during six days prior to the injection of histamine survived in many instances a dose of 800 mg of histamine per kilogram of body weight. The effectiveness of the parenteral administration of any given amount of cortical hormone to hypophysectomized rats probably varies to some degree with the total amount of cortical hormone which may still be available in the involuted adrenal cortex.

¹¹ Extract of adrenal cortex (Parke, Davis & Co.)

TABLE 5—*Effect of Repeated Injections of Adrenal Cortical Hormone on Natural Resistance of Hypophysectomized Adult Albino Rats to Histamine Poisoning**

Rat	Sex	Operative Procedure	Interval Between Operation and Injection of Adrenal Cortex, Histamine, Days	Weight at Operation, Gm	Weight at Beginning of Treatment with Cortical Extract, Gm	Weight at Time of Injection of Histamine, Gm	Amount of Histamine, Mg per Kg of Body Weight	Result	Comment
Rats Treated with Extract of Adrenal Cortex									
1	M	Complete hypophysectomy	7	205	183	196	700	Survived	
2	F	Complete hypophysectomy	14	191	178	180	800	Survived	
3	M	Complete hypophysectomy	13	271	221	233	800	Died	
4	M	Complete hypophysectomy	5	141	140	153	700	Survived	
5	M	Complete hypophysectomy	13	165	140	145	700	Died	
6	M	Complete hypophysectomy	14	174	150	161	800	Survived	
7	F	Complete hypophysectomy	11	154	125	132	800	Survived	Survived 2 days after injection of histamine
8	M	Complete hypophysectomy	11	157	138	130	700	Died	Marked atrophy of adrenals
9	M	Complete hypophysectomy	11	149	140	152	600	Survived	
Rats Not Treated with Extract of Adrenal Cortex									
11	F	Complete hypophysectomy	0	165	150	150	100	Died	
12	F	Complete hypophysectomy	12	152	117	100	100	Survived	
13	M	Complete hypophysectomy	13	205	170	170	700	Died	
14	F	Complete hypophysectomy	54	191	170	160	600	Died	
15	M	Complete hypophysectomy	50	179	170	200	200	Survived	
16	F	Complete hypophysectomy	11	152	124	124	200	Died	
17	F	Complete hypophysectomy	12	161	140	140	300	Died	
18	F	Complete hypophysectomy	15	180	158	158	400	Died	
19	M	Complete hypophysectomy	16	210	192	192	900	Survived	
20	F	Exposure of hypophysis without removal	0	212	196	196	600	Survived	
21	F	Normal control	14	185	165	165	900	Survived	
22	M	Normal control	12	160	185	185	900	Died	
23	F	Normal control	0	1,000	160	160	1,000	Died	
24	M	Normal control	0	1,000	190	190	1,000	Died	

* All treated rats received 0.5 cc of cortical hormone (equivalent to 20 Gm of whole cortex) twice daily over a period of six days prior to the injection of histamine

COMMENT

It has been demonstrated in previous communications and by the work of other investigators that removal of the adrenal glands is associated with marked depression in the natural resistance to toxins, poisons and bacterial and protozoan infections¹² It was observed that resistance to histamine in adrenalectomized rats¹¹ may drop to one eighth that of the normal animal, and it was suggested that histamine may be used as a gage of adrenal insufficiency

In subsequent work by Perla and Marmorston it was found that the level of resistance to histamine after adrenalectomy could be raised almost to that of the normal animal by the administration of extract of the adrenal cortex² containing the life-prolonging hormone of the adrenal cortex This suggested that the depression in the resistance to histamine after adrenalectomy was essentially due to loss of cortical function, particularly since administration of the medullary secretion, epinephrine, had only a slight effect in raising the resistance to histamine poisoning in adrenalectomized rats¹

The extensive work of Smith¹¹ and other investigators¹⁵ demonstrated that hypophysectomy in rats is followed by atrophy of the adrenal gland¹⁶ This suggested to us that a decrease in the resistance to histamine might follow hypophysectomy in rats

12 Perla David, and Marmorston, J Arch Path **16** 379, 1933

13 Marmorston-Gottesman, J, and Gottesman, J I Exper Med **47** 503, 1928

14 Smith, P E Anat Rec **32** 221, 1926, J A M A **88** 158, 1927, footnote 3

15 (a) Richter, C P, and Wislocki, G B Am J Physiol **95** 481, 1930 (b) Evans, H, Meyer, K, Simpson, M E, et al The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis, vol XI, Memoirs of the University of California, Berkeley, Calif, University of California, 1933 (c) Collip, J B, Selve, H, and Thomson, D Nature, London **131** 56, 1933

16 Similar observations have been described in the tadpole (Pigmentary Growth and Endocrine Disturbances Induced in the American Tadpole by the Early Ablation of the Pars Buccalis of the Hypophysis, American Anatomical Memoirs, no 11, Philadelphia, Wistar Institute of Anatomy and Biology, 1920, p 151 Atwell, W F Proc Soc Exper Biol & Med **29** 621, 1932), in the rabbit (Ikeda and Kusonoki, J Jap J Obst & Gynec **15** 213, 1932, Folia endocrinol japon **3** 34, 1927), and in the dog (Houssay, B A, and Sammartino, R Compt rend Soc de biol **114** 717, 1933) In man under conditions associated with hypofunction of the hypophysis and in Simmond's disease, the adrenal glands are atrophic (Falta, W Erkrankungen der Blutdrusen, Berlin, Julius Springer, 1913, Cushing, H, and Davidoff, L M The Pathological Findings in Four Autopsied Cases of Acromegaly with a Discussion of Their Significance, Monogr 22, Rockefeller Institute for Medical Research, 1927, p 1, Erdheim, J Beitr z path Anat u z allg Path **62** 302, 1916, and Simmonds, M Deutsche med Wchnschr **45** 482, 1919) The atrophy of the adrenal cortex in hypophysectomized rats may be repaired by daily homotransplants of hypophysis (Smith³), by the injection of

Hence the present studies were undertaken, and it was found, as anticipated, that a depression in the natural resistance of adult albino rats to histamine poisoning occurred after hypophysectomy¹⁷ The resistance was reduced to a point as low as one fifth that of normal rats, the period of greatest susceptibility to histamine poisoning occurring from about one to two weeks after operation In the later weeks the minimum lethal dose of histamine for hypophysectomized rats was increased to from about one third to one half that for the normal rat The decrease in resistance was associated with hemorrhage into the reticular zone or with atrophy of the cortex of the adrenals Partial hypophysectomy, in which the posterior lobe was destroyed but fragments of the anterior lobe remained, was also associated with a decrease in the natural resistance to histamine when involutional changes of the adrenal cortex occurred

That the decrease in resistance to histamine after hypophysectomy is associated with the removal of the anterior lobe and not with that of the posterior lobe is indicated by the fact that the rats in which the posterior lobe was completely removed but in which a large fragment of anterior lobe remained survived injections of histamine in nearly the same amounts as did normal animals, provided involutional changes were not present in the adrenal cortex

Since hypophysectomy results in atrophy of the adrenal cortex without producing noticeable changes in the adrenal medulla, the depression in natural resistance to histamine after this operation is probably not attributable to disturbance of medullary function but is probably associated with cortical atrophy induced by the withdrawal of the adrenotropic hormone of the anterior lobe of the hypophysis

This hypothesis is corroborated by the finding that administration of adrenal cortical hormone raised the natural resistance of hypophysectomized rats (just as it does of adrenalectomized rats²) to histamine poisoning, in some instances almost to the normal level

SUMMARY

The natural resistance to histamine was depressed in completely hypophysectomized rats from one to ten weeks after operation The minimum lethal dose was from one fifth to one third that for normal rats

potent extracts of the growth-regulating hormone (Evans, Myers and Simpson^{10b}) or of the adrenotropic hormone (Anderson, E M, Thomson, D L, and Collip, J B *Lancet* **2** 347, 1933) The administration of extract of the adrenal cortex in hypophysectomized rats does not prevent or repair the atrophy of the cortex (Shumacker, H B, and Firor, W M *Endocrinology* **18** 676, 1934)

17 It is interesting that, although hypophysectomized rats demonstrated a marked decrease in resistance to histamine, this depression was about one half as severe as that occurring after adrenalectomy in rats

This decrease in resistance was associated with hemorrhage into, or atrophy of, the inner zone of the cortex of the adrenal.

Rats in which the posterior lobe and most of the anterior lobe were removed showed a similar decrease in resistance. In these instances atrophic changes in the adrenal cortex occurred. When a large fragment of anterior lobe remained there was no depression of resistance to histamine and the adrenal glands were normal.

Repeated injections of large amounts of adrenal cortical hormone raised the natural resistance of totally hypophysectomized adult rats to histamine poisoning. In some instances the resistance was raised almost to the level of that of normal rats.

The decrease in natural resistance to histamine after hypophysectomy in the rat is probably secondary to the atrophic changes in the adrenal cortex induced by the withdrawal of the adrenotropic hormone of the anterior lobe.

PROTEOLYTIC ENZYMES OF MONOCYTIC AND POLYMORPHONUCLEAR PLEURAL EXUDATES

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With the development of the conception that the reticulo-endothelial system is the site of antibody production,¹ the attention of immunologists has been directed toward a more intensive study of the cellular defense mechanisms of the body. A reinvestigation of the phenomenon of inflammation from this point of view led Opie² and Menkin³ to conclude that this process is largely one of defense. Gay, Clark and Linton⁴ showed that there are definite differences in the defensive properties of the various types of cells in an exudate. Thus, only the monocytes (clasmatocytes) are concerned in protection against micro-organisms like the streptococcus and the pneumococcus. The polymorphonuclear cells, on the other hand, not only may be ineffective, but may actually serve as a culture medium for these bacteria. Observations were also made by Opie, Barker and Dochez⁵ and later by Jobling and Petersen⁶ on the enzymes of the cells of inflammatory exudates and on the rôle of the so-called enzyme-antienzyme balance in infection and immunity.

The development in recent years of more accurate physicochemical methods and of new points of view in the study of enzymes, particularly through the work of Northrop,⁷ Willstätter⁸ and Waldschmidt-Leitz⁹

From the Research Laboratories of the Mount Zion Hospital

1 Jaffe, R H. *Physiol Rev* **11** 277, 1931

2 Opie, E L. *J Immunol* **17** 329, 1929

3 Menkin, V. *Arch Path* **12** 802, 1931, *Arch Int Med* **48** 249, 1931

4 (a) Gay, F P, and Clark, A R. *Arch Path* **1** 847, 1926. (b) Gay, F P, Clark, A R, and Linton, R W. *ibid* **1** 857, 1926. (c) Linton, R W. *ibid* **6** 615, 1928

5 Opie, E L. *Physiol Rev* **2** 552, 1922, *J Exper Med* **7** 316, 1905, **8** 410 and 536, 1906, **9** 391, 414 and 515, 1907, **10** 419, 1908, *New York Path Soc* **7-8** 187, 1907, *Bull Johns Hopkins Hosp* **19** 115, 1908. Opie, E L, and Barker, B I. *J Exper Med* **9** 207, 1907, **10** 645, 1908, **11** 686, 1909. Opie, E L, Barker, B I, and Dochez, A R. *ibid* **13** 162, 1911. Barker, B I. *ibid* **10** 666, 1908. Dochez, A R. *ibid* **11** 718, 1909

6 Jobling, J W, and Petersen, W F. *Bull Johns Hopkins Hosp* **26** 356, 1915

7 Northrop, J H. *J Gen Physiol* **16** 41, 1932. Kunitz, M, and Northrop, J H. *J Gen Physiol* **17** 591, 1934

8 (a) Willstätter, R, Bamann, E, and Rohdewald, M. *Ztschr f physiol Chem* **188** 107, 1930. (b) Willstätter, R, and Rohdewald, M. *ibid* **204** 181, 1932

9 Waldschmidt-Leitz, E. *Physiol Rev* **11** 358, 1931

and their associates, suggested a reinvestigation of this subject. The present communication is concerned with the proteolytic enzymes of exudates of the monocytic and polymorphonuclear types which had been produced in the pleural cavities of normal rabbits. It is the first of a series of papers on the relation between the enzymic hydrolysis and the bacteriolysis accomplished by these exudates and on the possible effects of various bacterial toxins, carbohydrates and other substances as activators and inhibitors of these processes.

REVIEW OF THE LITERATURE

The investigations of Opie, Barker and Dochez revealed that polymorphonuclear cells obtained from pleural inflammatory exudates of dogs contain an enzyme, leukoprotease, which digests proteins in both neutral and alkaline reactions. Cells of the mononuclear series, on the other hand, have a lymphoprotease which acts only in weakly acid reaction. Husfeldt¹⁰ reported two proteinases, cathepsin and trypsin, in neutrophils which had been obtained from a man with myelogenous leukemia. The first of these represents the enzyme which is active during autolysis, it digests proteins from pH 3 to 7, the optimal digestion of casein and edestin is at pH 4.3 and 5.2, respectively. The second enzyme, leukocytic trypsin, begins to function at pH 4 and increases in activity with growing alkalinity of the solution. A third enzyme, dipeptidase, which is present in mixed leukocytes of normal human blood, digests the dipeptides, alanyl-glycine and leucyl-glycine, with optimal activity at pH 7.2 and 8.1, respectively, and the tripeptide, alanyl-glycyl-glycine, at pH 7.3. These peptidases were further subdivided by Waldschmidt-Leitz⁹ into a carboxy-polypeptidase and an amino-polypeptidase.

Kleinmann and Scharr¹¹ reported that hydrogen sulphide and hydrocyanic acid increase the proteolytic activity of the cathepsin of rabbit leukocytes, but only if gelatin is used as a substrate, no activation is observed with casein or edestin. The enzyme content of leukocytes varies somewhat with the species of animal used. Thus Willstätter, Bamann and Rohdewald^{8a} found the leukocytes of the dog to be richer in trypsin than those of the horse. Moreover, in the former, trypsin is fully active, while in the latter activation is necessary.

The proteolytic enzymes of serum were likewise investigated several years ago by Opie and his associates. More recently Stephan and Wohl¹² and Kleinmann and Scharr^{11b} have increased knowledge of this subject. Two proteinases were observed in the serums of rabbits and horses: a cathepsin associated with the globulin fraction and a tryptic enzyme bound to the albumin of the serum and liberated only when adsorbed on another protein such as casein. Enterokinase has no effect on either the bound or the free trypsin of serum. Heating to 56°C or adjusting the reaction to pH 5 or less¹³ causes serums to lose their antitryptic power.

METHODS OF INVESTIGATION

Monocytic exudates were produced in the pleural cavities of rabbits by the injection of light mineral oil or a liquid petrolatum, specific gravity from 0.88 to 0.89,

10 Husfeldt, E. *Ztschr. f. physiol. Chem.* **194** 137, 1931.

11 (a) Kleinmann, H., and Scharr, G. *Biochem. Ztschr.* **251** 275, 1932, (b) **252** 145, 1932.

12 Stephan, R., and Wohl, E. *Ztschr. f. d. ges. exper. Med.* **24** 391, 1921.

13 Weiss, C. *J. Infect. Dis.* **41** 467, 1927.

at 25 C¹⁴ After an interval of five days, the animals were exsanguinated by bleeding from the carotid arteries under ether anesthesia, and the exudates were removed by opening the thoracic cavity. Five rabbits usually yielded about 75 cc of exudate including about 45 cc of oil, which was discarded. The exudate was divided into two equal parts, one of which was immediately separated by centrifugation into cells and supernatant fluid, while the other was placed in the refrigerator and tested as "total exudate" on the following day. The sedimented cells were washed four times with physiologic solution of sodium chloride in order to remove traces of serum proteins, then extracted over night in distilled water at refrigerator temperature (10 C) and again centrifugated to remove the cellular residue or stroma. The latter was examined separately. The aqueous cellular extracts and the supernatant fluids were diluted with sufficient distilled water (about 96 cc) to provide 2 cc of enzyme material for each test tube.

Differential counts of the exudates made with the aid of Wright's stain and a supravital technic (neutral red used) showed about 90 per cent mononuclear cells, many of which were clasmatocytes. A few neutrophils were seen inside the cytoplasm of the monocytes, and a number of the cells contained oil in their cytoplasm. Several mesothelial cells and fibroblasts were also recognized. For comparison, polymorphonuclear exudates were produced by injecting a mixture of 5 per cent aleuronat and 3 per cent starch and removed after twenty-four hours. Differential counts of this exudate showed from 90 to 93 per cent polymorphonuclear cells and from 7 to 10 per cent clasmatocytes or histiocytes.

As substrate, 2 cc of 2 per cent iso-electric gelatin was employed. This quantity was adequate, since on complete hydrolysis its total combining capacity in the titration in the presence of formaldehyde is 36 cc of hundredth-normal sodium hydroxide, according to Cohn¹⁵. In some experiments a 2 per cent solution of purified casein was also used.

In order to determine the optimal p_H for autolysis or digestion, aliquot portions of each fraction of the exudate were placed into pyrex test tubes 25 by 150 mm in size and adjusted by means of either hydrochloric acid or sodium hydroxide to p_H 2, 3 and so on up to p_H 10 at intervals of 1 p_H unit with the aid of the glass electrode of de Eds¹⁶. No buffers were employed, since it was not desired to introduce substances which might interfere with enzyme action. The total volumes were adjusted with distilled water to 10 cc and the tubes placed in an air incubator at 37 C for twenty-four hours. Determinations of p_H made at this time revealed no serious changes, the alterations seldom exceeded 1 p_H unit. Filtrations were done by the method of Northrop¹⁷. After adding neutral formaldehyde, the material was adjusted to p_H 6, and the titration carried to p_H 9 by means of hundredth-normal sodium hydroxide. Standard color tubes were used as guides in determining the end-points. The titration values of material which had been previously boiled for thirty minutes in order to destroy the proteolytic enzymes were subtracted from the corresponding values for material which was boiled just before titration. In determining the digestion of gelatin by an enzyme, the values obtained for autolysis were subtracted from the corresponding uncorrected figures obtained by titration in the presence of formaldehyde.

14 Lucke, B., Strumia, M., Mudd, S., McCutcheon, M., and Mudd, E. B. H. *J. Immunol.* **24** 455, 1933.

15 Cohn, E. J. *Ergebn. d. Physiol.* **33** 781, 1931, tables on pp. 870 and 871.

16 de Eds, F. *Science* **78** 556, 1933.

17 Northrop, J. H. *J. Gen. Physiol.* **9** 767, 1926.

THE AUTOLYSIS OF PLEURAL EXUDATES

As seen in chart 1 *A*, an aqueous extract of rabbit monocytes of inflammatory origin contains a peptic enzyme which permits autolysis in acid reaction between (approximately) p_H 2 and 5, the optimum being at p_H 3. There is no evidence of cathepsin or of trypsin. An aqueous extract of polymorphonuclear cells (chart 2 *A*), on the other hand, shows activity in the region of p_H 3, 5.4 and 8, suggesting peptic catheptic and tryptic types of digestion (autolysis). The supernatant fluid of a monocytic exudate fixed immediately from its cellular elements (chart 1 *B*) shows tryptic digestion from about p_H 7 to 10, with an optimum at about p_H 8 and a slight amount of peptic action at p_H 3. The serous portion of a polymorphonuclear exudate (chart 2 *B*) behaves similarly but shows a little more peptic activity.

When an aqueous extract of monocytic cells is allowed to autolyze in the presence of the corresponding supernatant fluid, the former exerts strong inhibitory action on the latter from about p_H 10 to 7 (chart 1 *C*). A slight stimulation of cathepsin occurs between p_H 4.5 and 6.5. In the peptic range, between p_H 2 and 4 there is definite inhibitory action exerted by the supernatant fluid on the extract of the monocytes¹⁸. In the case of the polymorphonuclear cells, antitryptic action by the fluid is similarly evident. There is, however, inhibition of catheptic activity from p_H 5 to 7 with augmentation of peptic hydrolysis from p_H 2 to 4 (chart 2 *C*).

Since Gay and his co-workers discovered that the fluid separated from a pleural exudate after a delay of several hours loses its bactericidal power, it was of interest to ascertain how the proteolytic enzymes behave under similar circumstances. It was observed that when centrifugation of a polymorphonuclear exudate is delayed for twenty-four hours, the resulting fluid shows no cathepsin. Under similar circumstances the supernatant fluid of a monocytic exudate (table 1) acquires additional peptic activity, owing probably to autolysis of some of the cells.

When either a monocytic or a polymorphonuclear exudate was tested "whole," that is, without separation of leukocytes from the fluid, the autolysis accomplished in the regions of pepsin and cathepsin was greatly increased. The monocytic exudate showed, however, definite evidence of antitryptic action, since the level of the curve fell below that of the supernatant fluid or of the algebraic sum of the two constituents. The whole polymorphonuclear exudate did not show this phenomenon.

18 Hamburger, W. W. J. Exper. Med. 14 535, 1911.

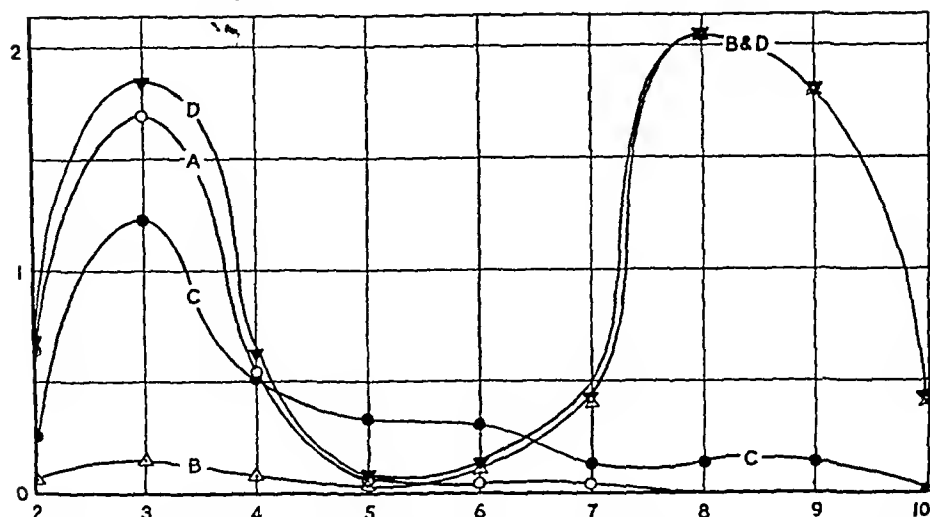


Chart 1—Autolysis of an inflammatory monocytic exudate at various pH values *A*, an aqueous extract of the cells, *B*, the supernatant fluid, *C*, both of these undergoing autolysis together (after being reunited), *D*, the algebraic sum of *A* and *B*, each undergoing autolysis separately. The numbers at the left represent values obtained by titration in the presence of formaldehyde, expressed in cubic centimeters of hundredth-normal sodium hydroxide. The numbers at the bottom represent the initial pH values.

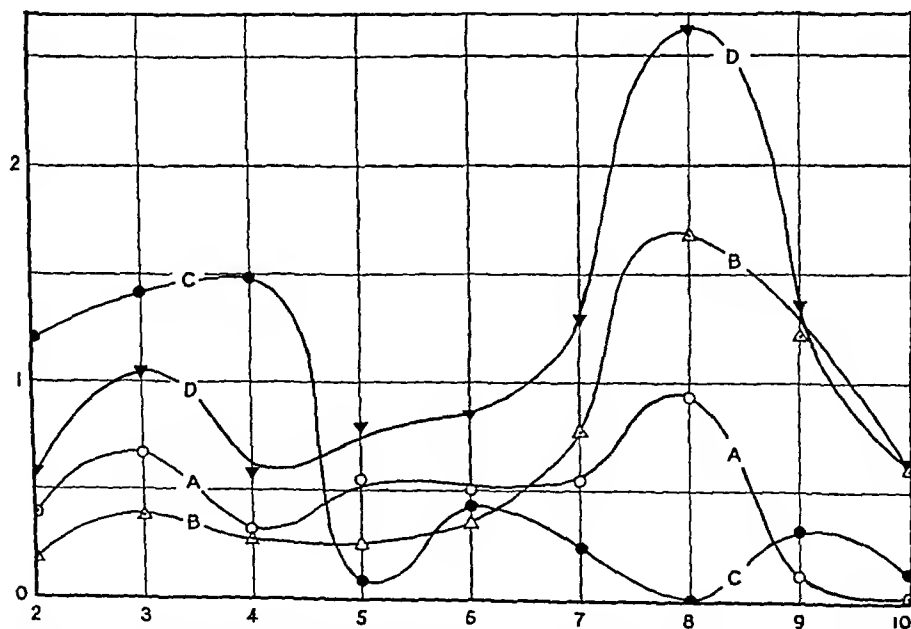


Chart 2—Autolysis of an inflammatory polymorphonuclear exudate at various pH values. See legend of chart 1 for explanation.

THE DIGESTION OF GELATIN BY PLEURAL EXUDATES, WITH
OBSERVATIONS ON RESYNTHESIS

The curves for hydrolysis of gelatin by aqueous extracts of cells of the monocytic and polymorphonuclear series are similar to those for autolysis (chart 3 *A*). Pepsin, cathepsin and trypsin are present in the latter and only pepsin in the former. Both supernatant fluids show tryptic activity but in the acid range they differ, in that the monocytic type shows absence of peptic enzyme while the polymorphonuclear is slightly active.

In the region between p_H 4 and 6 or 7 negative values in the titration in the presence of formaldehyde were observed with both types of supernatant fluid. The tubes contained gelatinous precipitates which

TABLE 1—*Autolysis of a Monocytic Exudate (Cells Removed After Twenty-Four Hours)*

Initial p_H *	Final p_H	Values Obtained by Titration in the Presence of 1 formaldehyde (Ce. of 100th Normal NaOH)		
		Total Exudate	Boiled Control	Enzyme Activity
2.0	2.1	1.14	0.80	0.34
3.0	3.4	1.86	0.76	1.10
4.0	4.1	1.03	0.59	0.44
5.0	5.1	0.53	0.48	0.05
6.0	6.0	0.45	0.42	0.03
7.0	7.0	0.68	0.45	0.23
8.0	7.7	1.27	0.53	2.74
9.0	7.9	1.11	0.79	2.64
10.0	8.7	2.31	0.89	1.42

* The original p_H of the material was 6.12

were soluble in strong alkali. In view of the work of Wasteneys and Borsook,¹⁹ Voegtlin²⁰ and others,²¹ these findings are tentatively accepted as evidence of reversed enzyme action or resynthesis (plastein formation).

It is also to be noted (chart 3 *C*) that in the presence of gelatin the monocytic supernatant fluid exerts no antitryptic action on the cell extract. This may be explained by the observation of Northrop²² that gelatin combines with the antitryptic or inhibitor substance. However,

19 Wasteneys, H., and Borsook, H. *Physiol Rev* **10** 110, 1930

20 Voegtlin, C. Maver, M. E., and Johnson, J. M. *J. Pharmacol. & Exper. Therap.* **48** 241, 1933

21 Blagowestschenski, A. W., and Jeremjew, G. W. *Biochem. Ztschr.* **270** 66, 1934

22 Northrop, J. H. *J. Gen. Physiol.* **4** 261, 1922

since the antitryptic action persists when gelatin is exposed to the combined action of polymorphonuclear cells and fluid, an alternate hypothesis is suggested, namely, that resynthesis and hydrolysis proceed simultaneously, the curves representing the algebraic sum of these two separate effects. It is also possible that the fluid of a polymorphonuclear exudate contains much more inhibitor substance than the aqueous extract of monocytes. Hence, the quantity of gelatin employed in the test may not have been sufficient to combine with all of the inhibitor substance present. This subject, however, requires further investigation.

When centrifugation of either a monocytic or a polymorphonuclear exudate is delayed for twenty-four hours, the resulting fluid shows absence of catheptic activity and of resynthesis. Tryptic digestion seems to be markedly increased, suggesting an inactivation of the inhibitor of trypsin or a release or activation of the enzyme. When centrifugation of a monocytic or polymorphonuclear exudate is entirely omitted

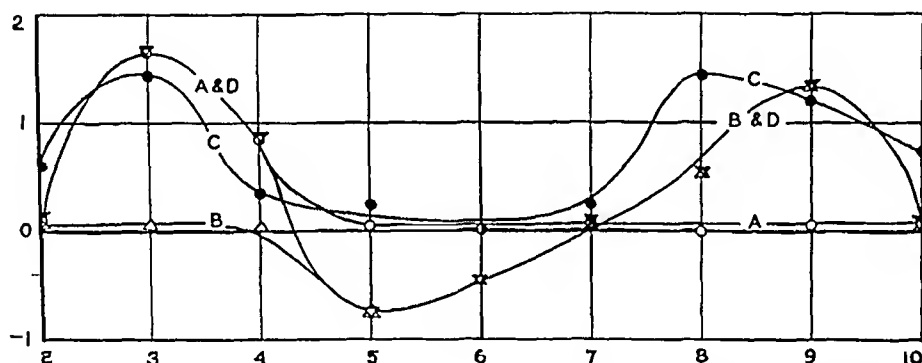


Chart 3—Digestion of gelatin by a monocytic exudate at various p_H values. See legend in chart 1 for explanation.

and the whole exudate is tested, cathepsin is present, but resynthesis is not evident. In the region between p_H 2 and 4 there is evidence of diminished peptic activity in the case of the monocytic exudate and of strong activation in the case of the polymorphonuclear type.

The amount of gelatin hydrolyzed by extracts of either polymorphonuclear or monocytic cells depends on the amount of enzyme supplied. Similarly the amount of resynthesis varies directly with the amount of fluid present. The quantity of gelatin available as substrate did not affect the extent of resynthesis, double the amount usually employed gave similar results. Hence the protein resynthesized is probably derived from the protein-split products of the inflammatory exudate. Since gelatin, but not casein, combines with the so-called inhibitory substance²² and helps to activate serum cathepsin,^{11b} it was interesting to find absence of resynthesis in the presence of casein.

A dipeptide, leucyl-glycine, was also exposed to the action of various fractions of a monocytic exudate at p_H 3, 5.5 and 8.5. Both the fluid

and the whole exudate hydrolyzed this dipeptide at p_H 8.5. At p_H 5.5 negative values were obtained by titration in the presence of formaldehyde with the former suggesting that resynthesis can occur in the presence of a dipeptide as well as in that of gelatin.

THE PRESENCE OF MUTUALLY ANTAGONISTIC LYO-ENZYMES AND DESMO-ENZYME IN MONOCYTES AND POLYMONUCLEAR CELLS

Willstätter, Bamann and Rohdewald²¹ demonstrated that the stroma remaining after extraction of leukocytes with glycerin is actively proteolytic, and designated this bound enzyme as "desmo-enzyme" while the extractable portion was termed "lyo-enzyme." In the present commun-

TABLE 2—*Mutual Inhibition of Leukocytic Lyo-Enzyme and Desmo-Enzyme*

Type of Cell	p_H	Preparation	Values Obtained by Titration in the Presence of 1 formaldehyde (Cc. of 100th Normal NaOH)		
			Autolysis	Digestion of Gelatin	Digestion of Casein
Monocytes	3.0	Stroma and cell extract combined	1.10	5.00	0.60
	3.0	Mathematical sum	4.75	4.10	3.20
	8.5	Stroma and cell extract combined	0.20	0.00	0.00
	8.5	Mathematical sum	0.15	0.05	0.10
Polymorpho nuclear cells	3.0	Stroma and cell extract combined	0.20	3.20	0.40
	3.0	Mathematical sum	1.70	3.50	4.50
	8.5	Stroma and cell extract combined	0.50	3.40	0.80
	8.5	Mathematical sum	2.65	2.90	3.35

cation we confirm and extend these observations. Our findings indicate that aqueous extracts of rabbit monocytes and polymorphonuclear cells contain more desmopepsin than lyopepsin. This was observed during autolysis as well as during digestion of casein or gelatin at p_H 3. The polymorphonuclear cells possess a comparatively large amount of desmo-enzyme of the tryptic type in their stroma. Since we have shown that rabbit monocytes contain no lyotrypsin, it was interesting to find absence also of desmotrypsin in these cells.

Willstätter's findings of a mutual antagonism between leukocytic lyotrypsin and desmotrypsin were also confirmed. The phenomenon was observed with monocytes at p_H 3 and with polymorphonuclear cells at both p_H 3 and 8.5. The substrates employed were casein or the proteins associated with enzyme itself (autolysis). With gelatin the antagonism was not apparent, possibly because this protein combines with the inhibiting substance, as suggested by Northrop²² (table 2).

COMMENT

It is a well known fact that during the development of an inflammatory reaction polymorphonuclear and monocytic phagocytes respond to the effects of the irritant. While the exact mechanism responsible for this phenomenon is not known, the work of Abramson²³ suggests that the migration of leukocytes to a point of injury may in part be dependent on the existence of a difference in potential between the injured and the relatively normal tissues. There is, moreover, an increase in the permeability of the blood vessels which permits the passage, in addition to the white blood cells, of an exudate containing serum proteins and fibrin. The digestion and absorption of these elements is accomplished by means of the proteolytic enzymes of the exudate. Owing to the concomitant appearance of substances which inhibit proteolysis, it is necessary to study their behavior and also the rôle of various activators and accelerators of enzyme action during the stages of an infection. Although several investigators, including Opie⁵ and Jobling, Petersen and Strouse,⁶ have made important contributions in this field, much still remains to be learned. For example, what is the relation between the conditions favoring enzymic digestion and the capacity of the exudate to destroy bacteria? Do bacterial toxins, autolysates, specific carbohydrates, etc., interfere with or stimulate the resolution of an exudate? Is it possible to regulate this mechanism at will during an infection? Our investigations were begun with the hope of answering some of these questions.

In the present publication we confirm and extend Opie's observations concerning important differences in the behavior of monocytes (clasmatocytes) and polymorphonuclear cells derived from pleural inflammatory exudates. Whereas the monocytes contain only one proteinase, pepsin, the polymorphonuclear cells have pepsin, cathepsin and trypsin. The supernatant serous fluids derived from these exudates also differ in that the former type contains a substance inhibiting peptic digestion while the latter contains one enhancing the peptic activity of the leukocytes. Moreover, in the case of the polymorphonuclear exudate, it is the supernatant fluid which inhibits the tryptic activity of the cells, while in that of a monocytic exudate it is the cells which inhibit the tryptic autolysis of the supernatant fluid.

In addition to this, there is an inhibitory mechanism which concerns the leukocytes themselves. This was first observed by Willstatter, Bamann and Rohdewald,⁸² who showed the presence of a mutually antagonistic extractable enzyme, or lyo-enzyme, and bound enzyme, or desmo-enzyme, in the leukocytes. This is confirmed, and their presence in both monocytes and polymorphonuclear cells of inflammatory exudates demonstrated.

²³ Abramson, H. A. *J. Exper. Med.* **46** 987, 1927.

Reference has been made to important differences which Gay and Clark¹⁸ observed in the bactericidal powers of these two types of exudates. Briefly, the monocytes can destroy streptococci and pneumococci, whereas the polymorphonuclear cells cannot. Exactly how this phenomenon is related to the differences in their enzyme action remains to be determined.

While the exact immunologic significance of the antienzyme substances in the blood or serous fluids has not been definitely established, the work of Wright¹⁹ suggests that the antityptic substance is related to the development of resistance to infection. It is necessary to confirm this and to determine whether the phenomenon has general application. Our observations to the effect that a cellular extract and its corresponding fluid at times inhibit each other's proteolytic activities while at other times they augment each other are strikingly similar to those of Mackie, Finkelstein and van Rooyen²⁰ who showed that the bactericidal power of a serum-leukocyte mixture may under certain conditions be greater than the sum of the separate activities of the serum per se and the leukocytes per se while under other conditions normal whole blood and serum-leukocyte mixtures are inferior to serum in bactericidal power.

During the course of this study it was also observed that while gelatin was being digested by the fluid of either a polymorphonuclear or a monocytic exudate there was a decrease in carboxylic groups as evidenced by negative values obtained by titration in the presence of a formaldehyde between p_{H} 4.5 and 5.5 (or 6). A similar phenomenon was observed during the digestion of a dipeptide, leucyl-glycine, by the fluid of the monocytic type of exudate. In view of the work of several authors,²⁶ this is suggestive of a resynthesis of the protein-split products present in the exudate due to a reversal of catheptic action. Whether a similar process may, under proper conditions, go on *in vivo* during an inflammatory process is under investigation in this laboratory. Should this phenomenon occur, it might help to explain some phases of the problem of delayed resolution in pneumonia as well as the production of adhesions and of organization.

A delay in separation of an exudate into its constituents (cells and fluid) causes an inactivation of the trypsin inhibitor or a release or an activation of trypsin. It also makes conditions unfavorable for resynthesis. Gay and Clark¹⁸ showed that a supernatant fluid loses its bac-

24 Wright, A. E. *Brit. M. J.* **2** 629, 1915.

25 Mackie, T. J., Finkelstein, M. H., and van Rooyen, C. E. *J. Hyg.* **32** 494, 1932; *J. Path. & Bact.* **39** 89, 1934.

26 Wasteneys and Borsook¹⁹ Blagowestschenski and Jeremejew²¹ Voegtlin, Mayer and Johnson²⁰

tericidal power if it is permitted to stay in contact with its cells for several hours before being tested. The question naturally arises. Do conditions which decrease bactericidal action also favor resynthesis in an inflammatory exudate, and hence delayed resolution?

It was pointed out in a foregoing paragraph that the optimal p_H for peptic digestion by monocytes or polymorphonuclear cells is 3. This is the p_H which Rous²⁷ found inside of these cells. The cells can maintain this intracellular acidity in spite of the alkalinity of the surrounding fluid by virtue of the presence of dissolved carbon dioxide²⁸. Since bacteria which are phagocytosed will find themselves in this acid reaction, this may be an important mechanism for the destruction of bacteria during inflammation. Mackie Finklestein and van Rooyen²⁵ have shown, moreover, that when bacterial toxins act on leukocytes, phagocytic activity may be destroyed without affecting bactericidal action, in fact, toxins may liberate the bactericidal substances from leukocytes. It is therefore tempting to conclude that conditions which favor peptic digestion by leukocytes also favor liberation of their bactericidal antibodies. This, however, is a subject for further investigation.

SUMMARY

Whereas monocytes of rabbits contain only one proteinase, pepsin, which is active from p_H 2 to 5, the optimal activity being shown at 3, the polymorphonuclears have pepsin, cathepsin and trypsin with optimal activity at p_H 3, 5.4 and 8, respectively. The serous portions of the exudates also differ in that the monocytic type contains a substance inhibiting peptic digestion by the leukocytes, while the polymorphonuclear type enhances this activity. The fluid of a polymorphonuclear exudate inhibits the tryptic activity of the corresponding cells, while cells of a monocytic exudate inhibit the tryptic activity of their fluid.

There is also an inhibitory mechanism which concerns the leukocytes themselves, observed by Willstatter, Bamann and Rohdewald, who showed the presence of mutually antagonistic extractable enzyme, or lyo-enzyme, and bound enzyme, or desmo-enzyme, in the leukocytes. This has been confirmed, and their presence in both monocytes and polymorphonuclear cells of inflammatory exudates has been demonstrated.

When gelatin was digested by the supernatant fluid of either a polymorphonuclear or a monocytic exudate, there was a decrease in carboxylic groups, as evidenced by negative values between p_H 4.5 and 5.5 (or 6) on titration in the presence of formaldehyde. A similar phenomenon was observed during the digestion of a dipeptide, leucylglycine, by the fluid of the monocytic type. In view of the work of

27 Rous, P. J. Exper. Med. **41** 379 and 403, 1925.

28 Jacobs, M. H. - Am. J. Physiol. **53** 457, 1920.

several authors, this is suggestive of a resynthesis of the protein-split products present in the exudate due to a reversal of catheptic action. Whether a similar process may, under proper conditions, go on in vivo during an inflammatory process is under investigation. Should this phenomenon occur, it might help to explain some phases of the problem of delayed resolution in pneumonia.

A delay in separation of an exudate into its constituents causes an inactivation of the trypsin inhibitor or a release or an activation of trypsin. It also makes conditions unfavorable for resynthesis. Gay and Clark showed that an exudate fluid loses its bactericidal power if it stays in contact with its cells for several hours. The question arises: Do conditions which decrease bactericidal power also favor resynthesis in an inflammatory exudate, and hence delay resolution? This remains to be determined.

DUPLICATION OF MECKEL'S DIVERTICULUM WITH OTHER CONGENITAL ANOMALIES

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ROCHESTER, MINN

Fitz¹ did not find a single case of duplication of Meckel's diverticulum reported in the literature prior to 1884, and I have not been able to find any since that date

Meckel's diverticulum varies considerably in size, shape and situation in the ileum. The usual length varies from about 2 to 10 cm, but larger ones, for example, that in the classic case reported by Moll,² have been seen. Tubular, conical, bulbous, irregular, hammer-shaped and even lobulated diverticula have been described. Cullen³ referred to a multilobulated diverticulum which was reported on by King in 1843 and to another diverticulum recorded by Gazin. The second one had two orifices, separated by a bridge of tissue, which opened into the intestine. Fitz mentioned the case reported by Hyrth in which the tip of a Meckel's diverticulum was divided into five parts. He described a diverticulum which had an incipient bifurcation of the tip. Nauwerck⁴ noted two diverticula of the ileum, he considered one a true Meckel's diverticulum and the other an acquired diverticulum which was produced by traction by a large mass of accessory pancreatic tissue.

Meckel's diverticulum is distinguished from an acquired diverticulum by the fact that it possesses the four coats of intestine similar to those of the adjacent portion of the ileum, that is, serosa, muscularis, submucosa and mucosa.

Frequently, a Meckel's diverticulum has a mesentery or a remnant of mesentery which extends along one side of the diverticulum for a variable distance.

Christie,⁵ Schaetz⁶ and other investigators noted that Meckel's diverticulum is frequently associated with other congenital anomalies. Christie observed this to be true in thirty-one of sixty-three cases.

Both the diverticula I am describing in this report were observed in the terminal portion of the ileum, they were antimesenteric in position,

1 Fitz, R H. Am J M Sc **88** 30, 1884

2 Moll, H H. Brit J Surg **14** 176, 1926

3 Cullen, T S. Embryology, Anatomy, and Diseases of the Umbilicus, Together with Disease of the Urachus, Philadelphia, W B Saunders Company, 1916

4 Nauwerck, C. Beitr z path Anat u z allg Path **12** 29, 1892

5 Christie, Amos. Am J Dis Child **42** 544, 1931

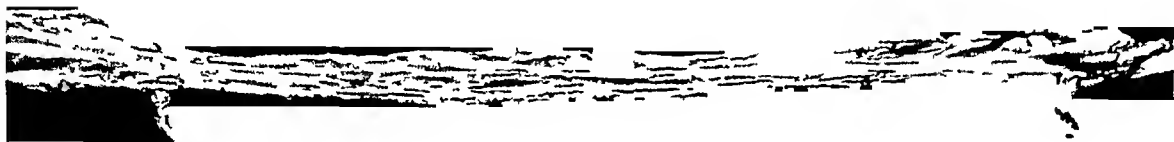
6 Schaetz, Georg. Beitr z path Anat u z allg Path **74** 115, 1925

possessed the same coats as the adjacent portion of the ileum and were associated with other congenital anomalies, and each had a mesentery of its own, which extended to the tip

REPORT OF A CASE

A hydrocephalic male fetus, 38 weeks of age, which had died in utero, was removed by craniotomy and extraction. A diverticulum, 2.5 cm in length and 1 cm in width, was situated 50 cm from the ileocecal valve on the antimesenteric portion of the ileum. A mesentery extended to the tip along one side of the diverticulum. Twenty centimeters proximal to this diverticulum was another, which also was situated on the antimesenteric border and was of the same length and width. It had a mesentery similar to that of the first. The diverticula are shown in the figure. Microscopic sections from the base, middle portion and tip of each diverticulum showed that they were similar to each other and to the adjacent portion of the ileum.

In addition to the diverticula that are described there were hydrocephalus, which was graded 3 on a basis of 4, patent ductus arteriosus with coarctation of the aorta, and nonlobulation of both lungs.



Terminal portion of the ileum, showing the two diverticula

COMMENT

It is difficult to explain the production of two Meckel's diverticula from the omphalomesenteric duct. I reasoned that in this case the process of atrophy of the omphalomesenteric duct was both altered and delayed, resulting in bifurcation of the tip similar to that which occurred in the case reported by Fitz. This bifurcation continued from the tip of the partially involuted omphalomesenteric duct to the base, with the result that the duct was divided neatly into two parts, each of which has a separate opening into the intestine. With the further growth of the intestinal tract each diverticulum grew independently and separated from its fellow by a distance equal to the increase in length of the intervening portion of intestine.

SUMMARY

A case is presented of duplication of Meckel's diverticulum in a 38 week old male hydrocephalic fetus. This condition was associated with anomalies of the nervous, cardiovascular and respiratory systems. A possible explanation of this anomaly has been offered.

Case Reports

THROMBOCYTOPENIC PURPURA ASSOCIATED WITH ADENOCARCINOMA OF THE STOMACH IN A YOUNG ADULT

GEORGE G STEBBINS, MD

AND

MARIE L CARNS, MD

MADISON, WIS

Lawrence and Mahoney¹ recently reviewed the literature on the association of carcinoma with thrombocytopenic purpura and reported one case of carcinoma of the stomach with extensive metastases and thrombocytopenia. They pointed out that carcinoma is practically always associated with a normal or an increased number of platelets in the peripheral blood and that the association of thrombocytopenia with carcinoma of the stomach is particularly uncommon. Because of the rareness of the finding of thrombocytopenic purpura associated with carcinoma of the stomach, especially in so young a person, the following case is reported.

REPORT OF A CASE

History—A woman, aged 21, was admitted to the hospital on July 3, 1933, with a chief complaint of "pain in the legs." She dated the onset of her illness to six weeks before, at which time she had intermittent sharp stabbing pain in her shoulders. A week before her admission the pain in the shoulders subsided, but she became nauseated and weak and vomited small amounts of dark blood. The following day the stools were black, and they had continued to be so until her admission. She had had no abdominal pain at any time. Two days before admission she first noted the "pain in the legs," which was her chief complaint. This pain she localized chiefly to the regions of the hip joints. The day before admission she noted ecchymotic areas on her arms and legs. Inventory by systems contributed little more of a pertinent nature. Her maximum weight was 140 pounds (63.5 Kg), nine years before, her average weight in recent years had been 135 pounds (61.2 Kg), and her present weight was 120 pounds (54.4 Kg). She specifically denied any additional symptoms referable to the gastro-intestinal tract other than recent anorexia and chronic constipation. Her past medical history showed that she had had only two diseases, measles and influenza. Tonsillectomy

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1 Lawrence, J. S., and Mahoney, E. B. *Am J Path* 10:383, 1934.

in 1928, appendectomy in 1929 and childbirth in 1931 had been characterized by uneventful recoveries. There was no familial history of hemorrhagic disease.

Examination—There were marked pallor of the skin and mucous membranes, rapid regular pulse and several small areas of subcutaneous hemorrhage over the lower extremities. There was no bleeding from the mucous membranes of the nose, mouth or throat. The spleen was not palpable. There were no abnormal cardiac findings other than tachycardia (rate, 110) and no abnormal objective findings in the region of the hip joints. The impression on admission was that the condition was purpura haemorrhagica (the type to be determined by laboratory studies).

The blood count on admission showed 50 per cent hemoglobin, 2,630,000 red cells and 11,600 white cells, with 83 per cent neutrophils, 3 per cent eosinophils and 14 per cent lymphocytes. The platelet count (direct method) was 30,000, the coagulation time (capillary pipet method), five minutes, and the bleeding time, three hours (plus). A flat roentgenogram of the pelvis showed nothing abnormal in the hip joints or pelvic bones.

Course—The patient appeared very ill and weak and complained so bitterly of pain in the hips on movement that only those laboratory studies deemed essential were carried out. Her course was progressively unfavorable despite all medication, including blood transfusions (citrated blood, from 200 to 500 cc) of which she had a total of eighteen. Small hemorrhagic areas appeared on the buccal mucous membranes, and she expectorated small amounts of bright red blood at intervals. Terminally she showed bilateral retinal hemorrhages and evidences of intracranial hemorrhage. The average level of the platelets during her hospitalization was 80,000. The terminal blood count (reported post mortem) showed 30 per cent hemoglobin, 1,410,000 red cells and 7,950 white cells with 35 per cent neutrophils, 2 per cent eosinophils and 63 per cent lymphocytes. No abnormal cells were reported. She died on August 8, thirty-five days after admission. The final clinical impression was that the condition was primary thrombocytopenic purpura.

Summary of Autopsy—*Macroscopic Examination*. Autopsy two hours after death revealed a poorly developed but fairly well nourished young white woman. The lips, gums and buccal membranes were very pale. A brownish area in the left conjunctiva was interpreted as a petechial hemorrhage. There were numerous purple petechiae on the upper extremities. A few scattered petechiae were found on the medial surface of each thigh and only an occasional one on the chest and abdomen. An oblique appendectomy scar was present in the right lower quadrant.

The peritoneal cavity contained 200 cc of a slightly blood-tinged fluid. The peritoneal surfaces were smooth, moist and glistening. There were no adhesions. (See the paragraph on the lymph nodes.)

The thorax contained no free fluid. There were no adhesions. The pericardial cavity contained 60 cc of a clear straw-colored fluid. The surfaces were smooth and moist.

The heart weighed 220 Gm. There were no abnormalities of the valves, endocardium, myocardium or coronary arteries. An occasional small petechia was noted in the epicardium.

The lungs were pale, voluminous and crepitant throughout. There were no consolidations or hemorrhages. The bronchi were clear. The blood vessels of the hilus contained no thrombi. The bronchial and mediastinal lymph nodes were enlarged and appeared to be producing pressure on the bronchi and blood vessels of the hilus. (See the paragraph on the lymph nodes.)

The spleen was enlarged, weighing 350 Gm. The capsule and cut surface were dark purple. The pulp was firm. The malpighian corpuscles were just visible.

The liver was enlarged and weighed 2,470 Gm. There were about a dozen small white nodules on the capsular surface up to 0.5 cm in diameter. Sectioning of the organ revealed more nodules of the same size. Congestion was noted around some of the nodules. The cut surface of the nodules appeared white and medullary.

The wall of the gallbladder was edematous and somewhat thickened. The organ was filled with bile. Numerous petechial hemorrhages were noted on the mucosal surface. The bile ducts were patent, but somewhat compressed by enlarged lymph nodes.

The pancreas and adrenals were of normal size and appearance.

The mucosa of the stomach was uniformly dark and congested in appearance. On the lesser curvature there was a round, slightly depressed and puckered area which measured 1.8 cm in diameter. The edges were not indurated, and there was little thickening in the base. The lesion appeared grossly like a healed peptic ulcer scar. The remainder of the alimentary tract was normal. The appendix had been removed.

The kidneys weighed 130 and 190 Gm each. When the capsule was stripped off an occasional pitted scar could be seen on the cortex. The cortex on the cut surface measured 7 mm, and it appeared somewhat opaque and cloudy.

The ureters, bladder, uterus and adnexa were of normal appearance. The aorta was smooth and elastic.

The bone marrow of the ribs and vertebral column appeared pale and rather dry.

Examination of the lymph nodes showed. The mediastinal and tracheobronchial lymph nodes were enlarged, firm and discrete. They ranged in size up to 2.5 cm in diameter. The nodes surrounded and appeared to be producing some external pressure on the trachea, bronchi and blood vessels of the hilus. There were similar nodes around the head of the pancreas, at the neck of the gallbladder, below the stomach and in the preaortic region. These nodes were all firm and discrete and ranged in size up to 4 cm in diameter. Some obstruction was produced about the neck of the gallbladder by the tumor nodules. The cut surface of some of the nodules, either mediastinal or abdominal, was medullary and hyperplastic in appearance, whereas others had areas of softening and yellow necrosis. Many of the larger nodes were soft and bloody in their central portions. No noticeable extensions were found to the pelvic bones or lumbar portion of the spine. However, the eighth thoracic vertebra was the site of a moderate-sized metastasis. The ribs and sternum revealed no evidence of metastatic growths.

Microscopic Examination. The heart, the adrenals, the urinary bladder and the uterus did not show any structural changes.

The alveolar walls of the lung were slightly thickened, and there was beginning smooth muscle hypertrophy. An occasional alveolus contained a clump of red blood cells. A most interesting feature was the distribution of the tumor cells in the blood vessels and lymphatics. The peribronchial lymphatic channels were distended and filled with tumor cells (fig 1). Other lymphatics were similarly filled, particularly those in the pleural region. Many of the blood vessels in the alveolar wall were filled with the tumor cells.

The pulp of the spleen was congested. The malpighian corpuscles were normal. There were primitive myeloid cells in the venous sinuses as well as occasional atypical tumor-like cells.

In the liver there were metastatic nodules of tumor growth. The adjacent liver cells were considerably shrunken and somewhat irregular in appearance. The Kupffer cells contained hemosiderin. Numerous radicals of the portal vein and the sinus spaces in many areas were filled with tumor cells. There were a number of foci of primitive-looking blood cells in some of the sinuses. One poorly-formed megalokaryocyte was noted.

The wall of the gallbladder was edematous.

Some of the islands of Langerhans were hypertrophied, and others appeared atrophic.



Fig 1—Photomicrograph of tissue from the lung. A bronchus is shown in the center, and the peribronchial lymphatic channels are distended with tumor cells.

The wall of the stomach at the site of the previously described puckered scar revealed a primary adenocarcinoma which was somewhat scirrhous in some areas. The glandular epithelium at the edge of the invasive growth was undergoing malignant degeneration. Lymphatics and smooth muscle in the deeper portions of the section were all being invaded. There was little connective tissue response. Many mitotic figures were noted (fig 2B).

The kidney tubules were somewhat swollen and granular. There were subcortical collections of lymphoid cells, and the subcortical glomeruli were becoming obliterated and hyalinized.

The lymphatics in the ovary were filled with tumor cells, but no perilymphatic invasion had occurred. There were areas of necrosis and hyalinization.

The lymph nodes were largely composed of tumor cells which were actively growing. There were small areas of necrosis and hemorrhage. Many mitotic figures were noted.

The bone marrow of the rib had an exhausted sclerotic appearance. The hematopoietic centers were appreciably reduced in numbers. There were no megalokaryocytes. A considerable amount of hemorrhage had occurred. Recent hemorrhages as well as areas of organizing hemorrhage were apparent. The main picture was that of sclerosis of the marrow. These scars were interpreted as areas of previous hemorrhage which had since become organized and replaced by connective tissue.

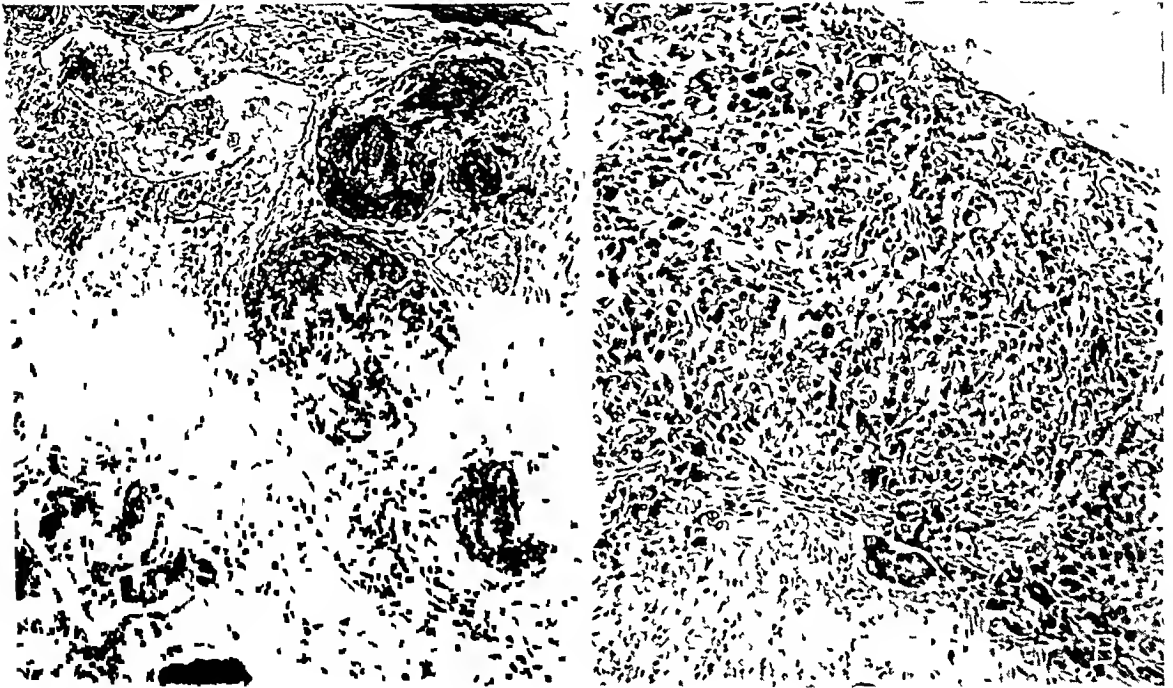


Fig 2—*A*, photomicrograph of bone marrow of the rib which reveals markedly reduced hematopoietic centers, hyaline thrombi on the right, areas of hemorrhage and venous sinuses in the left lower corner filled with tumor cells. *B*, photomicrograph showing primary adenocarcinoma of the stomach, tumor cells are seen in the lymphatics.

The arterial capillaries stood open and contained only red cells, but the thin-walled venous sinuses were filled with tumor cells. It seemed probable that behind the blocked sinusoids hemorrhages had occurred. Many of the sinusoids were filled with thrombi, some of which were becoming hyalinized. Only an occasional tumor cell was found outside the sinuses (fig 2*A*).

Sections from the vertebral metastasis revealed these pictures. One had tumor cells filling most of the sinuses and only an occasional small extravascular extension, another area revealed numerous patches of connective tissue scars. The major growth of tumor cells was in the sinuses.

SUMMARY

An interesting feature of this case was the predilection of the tumor cells for the lymphatics, lymph nodes and venous sinuses. The cells from the small primary gastric carcinoma readily invaded the lymphatics and gave rise to the large number of metastases to the lymph nodes noted in the mediastinum and peritoneal cavity. Lymphatic involvement in organs was particularly noted in the ovaries and lungs. Not a metastasis was found in the lungs outside the pulmonary lymphatics, which were widely distended especially in the peribronchial regions. The liver had a number of metastatic tumor nodules, and in addition numerous radicals of the portal vein and liver sinusoids were filled with tumor cells.

The venous sinuses of the bone marrow were filled with the tumor cells. It is our belief that the marked invasion and proliferation in these sinuses played a leading rôle in the changes produced in the peripheral blood picture. The exhausted appearance of the marrow with the marked reduction of hematopoietic centers and the apparent absence of megalokaryocytes would account for the reduction in hemoglobin and red blood cells as well as the diminution in the number of platelets. It is of interest in this connection that in the similar case reported by Lawrence and Mahoney¹ the marrow showed an approximately normal number of morphologically normal megalokaryocytes.

SPIROCHETAL ABSCESS OF THE LIVER

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Although spirochetes and fusiform bacilli and other associated organisms are commonly found in infections of the mouth (such as fusospirochetal angina, noma, pyorrhea alveolaris and periapical infections), in infections of the genital organs, intestinal tract and respiratory tract and, less commonly, in those of the eye, ear, nose, meninges and brain, their occurrence in abscess of the liver, osteomyelitis of the cranial bones and lesions of the axillary glands is rare

In 1926 Von Glahn¹ reported that he had found argentophilic microorganisms in two cases of supposedly sterile hepatic abscesses. The demonstration was made by the silver impregnation method of Levaditi. The spirilla observed in the first case stained intensely. They varied in form: some were long and undulating, containing at times as many as nine half convolutions, others were not so definitely convoluted but were curved and slightly twisted. The ends were rounded. In the second case, large masses of long, slender bacilli were seen. Many of these organisms were sharply curved, others were slightly curved, but none had convolutions similar to those described in the first case. No dark-field examinations were made. Zinserling² observed metastatic abscesses of the liver and kidney in a case of fusospirochetal infection of a carcinoma of the stomach, and Smith³ observed metastatic abscesses of the liver, kidney and spleen and also a metastatic fusospirochetal abscess of the femur following an operation on a fusospirochetal abscess of the lungs. Considering that hepatic abscesses have been described as sterile in about one half or more of the cases reported (Rolleston,⁴ Talbot,⁵ Elsberg⁶ and Giordano⁷), we agree with Von Glahn that more careful bacteriologic examinations should be made in cases in which no bacteria or actinomycetes appear to be present in the abscesses.

REPORT OF A CASE

A white man, aged 40, was admitted to the Lakeside Hospital on June 9, 1931, complaining of pain in the chest and swelling of joints of the fingers, wrists, knees

From the Institute of Pathology, Western Reserve University, and the University Hospitals

1 Von Glahn, W C. Proc New York Path Soc **26** 97, 1926

2 Zinserling, W D. Ueber die fuso-spirochätose Gangran und einige Prozesse vorzugsweise bei Kinder, Jena, Gustav Fischer, 1928

3 Smith, D T. Oral Spirochetes and Related Organisms in Fuso-Spirochetal Disease, Baltimore, Williams & Wilkins Company, 1932

4 Rolleston, H D. Diseases of the Liver, Gall-Bladder and Bile-Ducts, New York, The MacMillan Company, 1912

5 Talbot, P. Brit M J **2** 375, 1919

6 Elsberg, C A. Ann Surg **44** 217, 1906

7 Giordano, quoted from Davidsohn, C. Virchows Arch f path Anat **171** 523, 1903

and ankles. He gave a history of having had influenza followed by pneumonia, hemoptysis and the coughing up of large amounts of foul-smelling sputum. Other features of the past history and the familial history were irrelevant. At the hospital he showed marked pallor of the skin and mucous membranes. Respiration was rapid and shallow but not labored. There was a fetid odor to the breath. The teeth were in poor condition, and the gums were retracted. The head, ears and nose were normal. The right side of the chest was flattened anteriorly, with a posterior bulge beneath the scapula. The respiratory excursion on the right was somewhat restricted, and dullness to percussion at the base of the right lung with elevation of the breath sounds was noted. Rales occurred at the end of inspiration. Just beneath the angle of the scapula on the right, amphoric breathing was heard. The heart was not enlarged, but the beats were rapid, 110 per minute, and of fair quality. No murmurs were heard. The abdomen and genitalia were normal. There was clubbing of the fingers. The joints and the wrists, fingers, elbows, knees and ankles were swollen, with increased local heat and redness. The white blood cell count was 13,000, the erythrocyte count, 3,600,000, and the hemoglobin, 70 per cent. The Wassermann reaction was negative. The sputum showed a mixed flora but no spirochetes or acid-fast forms. The patient remained in the medical service seventy-four days, and during this time his sputum showed spirochetes and fusiform bacilli. Postural drainage was maintained, and five injections of nearsphenamine were administered. Bronchoscopy showed nothing unusual. A rib resection was performed on the level of the angle of the scapula, and the abscess was drained. Following irrigation of the wound with surgical solution of chlorinated soda and eight days prior to death, a positive blood culture (*Streptococcus gamma*) developed and signs of a fibrinous pleurisy appeared at the base of the left lung.

The clinical diagnosis was abscess of the middle lobe of the right lung, bronchopneumonia, fibrous pleurisy and septicemia (*Str. gamma*).

Autopsy.—Only the significant observations in the lungs and liver will be dealt with.

The cut surfaces of the upper lobe of the left lung revealed two adjacent spherical cavities near the periphery, measuring 1.5 by 2 cm. The walls of the cavities were thin, soft and structureless. They contained a dark green material, and no direct communication with the respiratory passages was observed. The overlying pleura was thickened.

The right lung appeared larger than normal and showed a considerable increase in weight. The pleural surface over the middle lobe, over the upper portion of the lower lobe and over the lower portion of the upper lobe appeared thickened, gray and fibrous, and showed numerous fibrous tags and bands. Cut surfaces revealed the middle lobe to be of greatly increased density, to contain a spherical cavity about 2.5 cm. in diameter, and to have a fibrous wall from 2 to 3 mm. in thickness. The cavity contained a thick, yellow, semifluid material, and its lining appeared to be structureless. The cut surface immediately surrounding this cavity showed increased density, appeared gray and fibrous and contained a small group of irregular areas of softening, varying in width up to 4 mm. These areas were in direct communication with the sinus in which the rubber drainage tube was inserted.

There was no communication between the thoracic and abdominal cavities.

On the superior aspect of the right lobe of the liver, at about the middle portion, the capsule was thinned out and showed a circular area (cavity) of dark green color, approximately 8 cm. in diameter. When this was opened, a thick,

yellow fluid having a foul odor escaped. The wall of the cavity was 4 mm in thickness, dark gray and soft.

Pathologic Diagnosis—Gangrenous bronchiectasis of the right lung and gangrene of the liver, chronic interstitial pneumonia, the wound of a surgical incision of the right thoracic wall with a sinus leading to the middle lobe of the right lung, acute fibroserous pleuritis of the left side, chronic fibrous pleuritis of the right side, acute fibrous pericarditis, acute hyperplasia of the spleen, cloudy swelling and passive hyperemia of the kidneys.

Bacteriologic Examination—Pus from the left lung contained fusiform bacilli, spirochetes, rods, cocci and pus cells. Some of the spirochetes observed by dark-field illumination and Fontana's stain appeared to be large and wavy (*Spirochaeta buccalis*) and others finer, wavy or closely coiled (*Spirochaeta Vincenti*). In the hemorrhagic exudate from the right lung masses of spirochetes of the same types were seen, in addition to fusiform bacilli and other bacilli of various sizes, cocci and pus cells.

The pus from the liver was yellow and showed masses of pus cells, vibrios and cocci. No spirochetes, typical fusiform bacilli or cysts were seen. The cavity of the liver was washed out with physiologic solution of sodium chloride, and a piece of hepatic tissue at the base of the ulcer was taken out, ground up and examined by dark-field illumination, and smears stained by Fontana's and Howell's⁸ methods. By these methods the spirochetes predominated.

The spirochetes were fine, and the majority appeared to be well coiled, showing an average of from seven to ten coils. Some were closely coiled, others loosely coiled. By dark-field illumination they appeared to be actively motile. The more closely coiled forms gave the appearance of *Treponema dentium*, although the majority appeared to be of the type of *S. Vincenti*. In the hepatic tissue stained by Fontana's method, they appeared in the intercellular spaces. Cultures on numerous mediums containing serum, ascitic fluid or hydrocele fluid and inoculations into traumatized testicles of rabbits failed to propagate them. The blood culture yielded *Str. gamma*.

SUMMARY

A case of spirochetal gangrene of the lungs and liver is described. The organisms in the hepatic tissue were discovered by dark-field examination of material from the base of the hepatic abscess, and the observation was confirmed when the hepatic tissue was stained by Fontana's method. No direct connection existed between the abscess of the liver and the abscesses of the lungs. The abscess of the lung was therefore considered to be of metastatic origin.

⁸ Howell's method of staining employs 1 per cent potassium permanganate and methyl violet 6B.

Laboratory Methods and Technical Notes

DI-NITROSOIRESORCINOL—A NEW SPECIFIC STAIN FOR IRON IN TISSUES

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The demonstration of compounds of iron, such as hemosiderin and various other allied iron compounds in tissues, has changed but little in technic in the last seventy years, and at present various modifications of the prussian blue reaction are employed almost to the exclusion of other methods. In the course of work on the recognition of iron in various organs it was found that all the present methods are wanting in one or more respects, and a search was made for new specific stains for iron. Many of these methods, such as the use of a sulphide lack specificity and fail to distinguish iron pigments from coal dust, silver salts and other dark materials and yet are superior to the specific ferrocyanide reactions which are relatively much less sensitive. This defect in sensitivity was conclusively demonstrated by testing the various methods on serial sections of iron-containing tissue and noting the variation in the amount of demonstrable pigment. The impermanent nature of the prussian blue reaction is also an objection, because in experimental work over a period of years marked fading was noted in slides less than two years old.

In an effort to find a more satisfactory stain for iron various reagents which gave a specific color reaction in colorimetric and metallurgic chemical analyses were used on iron pigment-containing tissue. Alcoholic and aqueous solutions of these chemicals were tested for their reactions to iron on sections from tissues fixed in formaldehyde and mounted in paraffin. In some instances the sections were first sensitized by a quick immersion in a solution of ammonium sulphide. The following specific reagents for the detection of iron were used: acetylacetone, an alcoholic solution of di-methylglyoxime, ammonium phenylnitrosohydroxylamine, alloxantin, thioglycolic acid and di-nitrosoiresorcinol. The last mentioned compound was found to be the only satisfactory stain. Its formula after drying, according to Orndorff and Nichols,¹ is $C_6H_2O_2(NO)_2H_2$, when it occurs as 2, 4, di-nitrosoiresorcinol, the formula is $(C_6H_2(N)_2)(CH)_2 \cdot 2H_2O$, according to Rosenthaler.² It is a dark brown powder, or it may occur as yellow-brown lamellae.

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1 Orndorff, W R, and Nichols, M L J Am Chem Soc **45** 1536, 1923

2 Rosenthaler, L, in Margosches, B M Die chemische Analyse, Stuttgart, Ferdinand Enke, 1923, 1920, p 483

which detonate at 115 C, according to Meyer and Jacobsen,³ or at 142 C, according to Rosenthaler Fitz,⁴ in 1875, first mentioned the formation of a green coloration or lake with ferric chloride. Di-nitrosoresorcinol is soluble with difficulty in cold water and alcohol, but is soluble in ether, benzene, toluene and chloroform. For the detection of iron the solution should be neutral.

It was noted in the work with this stain that if the tissue was first sensitized by a mere dip in ammonium sulphide the stain was much more satisfactory and the non stained rich green, moreover, if the slide was allowed to remain in the solution a few hours or overnight a pleasing brown counterstain resulted. The delicacy of this reaction is probably due to the ability of the new reagent to render minute quantities of iron visible, for it is evident that the sulphide renders the iron sensitive for the characteristic color reaction with the specific reagent. Potassium cyanide can be employed as a sensitizer, but it is not as satisfactory as the sulphide.

The reagent appears to be more effective after it has aged for a few days, this is possibly due to its relative insolubility. It seemed feasible to maintain a layer of the undissolved reagent in the bottom of the jar to insure saturation. The solution remains apparently unaltered and stains well after two years of almost constant use, although it becomes darker.

The tissue, after fixation in formaldehyde, is blocked in paraffin, sectioned and mounted on slides, after drying and removal of the paraffin the sections are stained by the following method:

- 1 Place the section in a jar containing a dilute (30 per cent) solution of ammonium sulphide for one minute

- 2 Rinse in water

- 3 Place in a staining jar containing a saturated aqueous solution of di-nitroso-resorcinol⁵ for from six to twenty hours, depending on the depth of the counterstain desired. (If an alcoholic solution is desired use a 3 per cent concentration in 50 per cent alcohol.)

- 4 Wash in water or dilute alcohol

- 5 Run through graded alcohols, carboxylol and xylol as usual

- 6 Mount in balsam

The sections appear brilliant, sharp dark green against a rich brown background. The cellular outline is clear, and the pigment granules do not fade to brownish red as in old slides stained with prussian blue. Slides which I stained in February 1930, over five years ago, still retain their pristine brilliance, sections stained at the same time with prussian blue from a block in the same series have a faded and unsat-

³ Meyer, V, and Jacobsen, P. *Lehrbuch der organischen Chemie*, Berlin, W de Gruyter & Co, 1902, vol 2, p 464

⁴ Fitz. *Ber d deutsch chem Gesellsch* 8 631, 1875, quoted by Orndorff and Nichols¹

⁵ Di-nitrosoresorcinol used in this method was obtained from the Division of Organic Chemicals of the Eastman Kodak Co, Rochester, N Y

satisfactory appearance. If the usual red counterstain is desired, the time given the third step in the process can be decreased to one hour or less.

The method outlined compares in specificity and sensitivity with any of the older methods, and unless speed is essential the counterstain will be found satisfactory. Except for the dip in the sensitizing sulphide, the process is reduced to one stage. The permanence of the stain, in contrast to that obtained by the contemporary methods in vogue, should warrant its use if the slides are to be kept for future reference.

General Review

EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS AND ITS RELATIONSHIP TO HUMAN ARTERIOSCLEROSIS

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BALTIMORE

(Concluded from page 124)

VIII ETIOLOGY AND PATHOGENESIS OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN RABBITS

The existing information concerning experimental cholesterol arteriosclerosis has been outlined in the survey of the literature presented in the preceding sections of this paper. More information on many points could be desired, and it is evident that a number of ideas which have found acceptance in certain quarters lack the substantiation of thorough experimental proof. Nevertheless, the evidence which may be regarded as reasonably well established is sufficient to serve as a basis for some deductions as to the etiology and pathogenesis of this experimental disease.

It will be recalled that it has been possible only in rabbits and guinea-pigs to show at all clearly that cholesterol feeding is capable of producing lesions in the arteries. So far as guinea-pigs are concerned, not much more than this is known. It is true that the arterial lesions which develop in guinea-pigs following the prolonged feeding of cholesterol-rich diets are similar to those found in rabbits treated in the same way, and it might be inferred from this fact that the manner of development of the lesions is practically identical in the two species of animals. However, so little is known concerning experimental cholesterol arteriosclerosis in guinea-pigs that nothing would be gained at present through an attempt to discuss the pathogenesis of the disease in these animals. Accordingly, this subject will be excluded from further consideration here, and the following discussion will be limited to the etiology and pathogenesis of experimental cholesterol arteriosclerosis in rabbits.

Before the importance of cholesterol in the diet was recognized, the earlier investigators² thought that the fatty arterial lesions which they had produced in rabbits were the result of a "fatty degeneration" of the arteries due to the toxic effects of the animal proteins in the diets

(2) 47, 65, 66, 147, 148

which they had used, and to the action of certain "mechanical" factors. Later, the production of similar lesions by the feeding of cholesterol alone or of cholesterol in oil showed that the proteins were not of essential importance, and attention was shifted to the rôle of cholesterol in the etiology and pathogenesis of the arterial lesions. It was soon realized that all of the lipid material which appeared in the walls of the arteries could not arise from the local breakdown of tissue, and it became necessary to account for its arrival from elsewhere.

The possibility was suggested that the progressive deposition of doubly refractive lipoids in the liver, spleen, bone marrow, suprarenal cortex and other tissues and finally the appearance of lipoids in the walls of the arteries represented a series of compensatory processes designed to relieve the blood stream of an excessive load of cholesterol which could not be handled adequately by the excretory apparatus.¹ This explanation has not been accepted widely because it neglects the fact that the deposits of cholesterol in the animal body occur in two distinct ways. The lipoids which appear in the cells of the suprarenal cortex and of the liver and in the cells of the reticulo-endothelial system seem to be taken up by these cells directly from the blood stream. But another kind of deposit occurs in which the lipoids are first precipitated in relation to the intercellular ground substance of tissues and may then be taken up secondarily by cells which subsequently are stirred into activity. These two processes are essentially different. The first represents a normal function which is exaggerated under the conditions of the experiments. So far as it assists in reducing the level of the blood cholesterol by temporary storage or otherwise, it may be considered as compensatory.² However, the deposition of cholesterol and of other lipoids in intercellular materials is a very different process, not acting under normal conditions and not to be regarded as compensatory, but rather as definitely pathologic. It is this process which must be explained to account for the appearance of fatty deposits in the arteries of cholesterol-fed rabbits.

The theory of the pathogenesis of experimental cholesterol arteriosclerosis which has found the greatest favor is a modification of the so-called "infiltration" or "imbibition" theory. The chief proponents of this theory, as it is applied to experimental cholesterol arteriosclerosis, have been Anitschkow and his associates. Anitschkow's most recent statement of his views¹² represents the epitome of all the ideas which he had previously expressed, and consequently only this publication need be drawn on for the following resume of the "infiltration" theory.

According to this theory, the lipoids which make their appearance in the arterial wall do not originate from "degeneration" of the tissue,

but infiltrate or impregnate it from the blood stream. The wall of the vessel is regarded as being perfectly normal and unimpaired in structure prior to the advent of lipid substances. The subsequent proliferation in the intima is viewed as the result of the presence of precipitated cholesterol, the precipitation of lipoids being the primary event. The lipoids in colloidal state enter the intima from the lumen of the artery with the "nutritive lymph stream" which normally seeps through the entire thickness of the wall of the vessel and is then carried off by the lymph channels or veins of the adventitia. During this slow permeation of the arterial wall, the "lymph," which presumably carries an increased concentration of lipoids, has to penetrate the ground substance and the latter accordingly becomes impregnated with fatty substances. The cholesterol and associated lipoids undergo "flocculent precipitation" during this slow passage, and they appear in the ground substance as stainable particles. In explaining the precipitation of the lipoids, great importance is attached to the normal physicochemical properties of the ground substance and to the slowness with which the "lymph" permeates the arterial wall. The usual preponderance of lipid deposit in the intima is accounted for by the resistance offered to the onflow of the "nutritive lymph stream" by the internal elastic lamina. The consequent slowing of the "lymph" stream is supposed to favor the precipitation of lipoids which therefore appear first and most abundantly in the intima.²¹

(a¹) In speaking of the pathogenesis of human arteriosclerosis, Rosenthal (*Arch Path* 18 660, 1934) recently added to the infiltration theory as here stated a further elaboration, the essential ideas of which are contained in the following quotation: "The serum of the blood stream infiltrating through the inner two thirds of the aorta may show a hindering of its lipoids at the elastic barriers. With contraction of the vessel in diastole there is an expression of this substance.

The lipid deposit is dependent on the disproportion of the infiltration over expression under normal conditions. It is for this reason that muscular arteries, which have markedly developed internal elastic membranes that contract more vigorously than the other constituent parts of the vessel, express the lipoids more efficiently." In this highly theoretical statement, there is an unfortunate confusion between the infiltrating, lipid-containing fluid, on the one hand, and the lipoids which may be precipitated from it in the form of a deposit, on the other. The fundamental question of the actual cause of the deposition or precipitation of lipoids from the colloidal state in which they exist in the infiltrating fluid is not touched on. The hypothetical failure of diastolic recoil to force infiltrating fluid out of the arterial wall could lead only to edema of the tissue, and so long as the lipoids remained in colloidal suspension they could not accumulate in one place. The precipitation of lipoids remains entirely unexplained. As to the "expression" of lipoids when once they have been precipitated in the form of a deposit, the idea that the mere mechanical pressure exerted by the diastolic contraction of any artery could remove the lipoids by forcing them back into the blood stream or back into solution in the tissue fluid seems positively fantastic.

From all of this, it is obvious that only one deviation from the normal condition is conceived as a necessary factor in the precipitation of lipoids, namely, an increased concentration of lipoids in the "nutritive lymph stream," a condition which presumably follows as a result of the hyperlipemia occasioned by cholesterol feeding. Up to this point, however, no explanation has been offered to account for the patchy distribution of the fatty deposits. If the function of the "nutritive lymph stream" is to nourish the inner layers of the wall of the vessel, it must permeate all parts of the intimal surface, and therefore lipoid deposits should occur diffusely over the whole extent of the artery. This difficulty has been obviated by the postulation of normal local differences in the permeability of the arterial walls. Support for this idea has been sought in some experimental work which may be mentioned briefly.

The conception of the normal flow of colloid-containing fluid from the lumen of the vessel into its walls is based on observations on the behavior of colloidal dyes, such as trypan blue, when they are introduced into the blood stream^b. Shortly after trypan blue is injected, the walls of the arteries become stained. In the aorta, certain areas are stained more deeply than the remaining parts, and these areas correspond fairly well with the localities in which fatty deposits occur most frequently in experimental cholesterol arteriosclerosis. Anitschkow¹² believes that most of the dye enters the wall of the aorta from its lumen, and that the lining endothelium of the aorta is just as permeable to colloids as are the capillaries. The more deeply stained areas are interpreted as indicating localities in which the outflow of the "nutritive lymph stream" from the lumen is normally more pronounced than elsewhere. The more abundant flow of lipoid-containing "lymph" through such local areas is offered as an explanation of the corresponding localization of fatty deposits in the aortas of cholesterol-fed rabbits,^c although such an explanation is entirely inconsistent with the conception that the slowness of a flow of the centrifugal "lymph" stream is of importance in the precipitation of lipoids.

I have repeated these experiments with the staining of the arteries of rabbits by the intravenous injection of trypan blue,¹⁴ and the results show clearly that, although the lining endothelium of the aorta is slightly permeable to the dye, it is not nearly so permeable as are the vasa vasorum. Furthermore, the permeability of the aortic lining is everywhere uniform. The more deeply stained areas seen on the intimal surface of the aorta are due to the escape of dye from the capillaries of the vasa vasorum, which have been shown by Robertson¹²⁵ to be much more abundant in these localities. The trypan blue deposited in the adventitia and outer layers of the media is visible on the intimal

(b) 10, 12, 55, 57, 83, 115, 120

(c) 55, 57, 115

surface because of the thinness and translucency of the rabbit's aorta. This fact apparently has led to the mistaken idea that the dye had entered from the intimal side.

These observations preclude the possibility of explaining the localized character of the lesions of experimental cholesterol arteriosclerosis on the basis of the "infiltration" theory as it is stated by Anitschkow. This fact, in turn, throws serious doubts on the adequacy of the explanation offered for the precipitation of lipoids in the arterial walls, an explanation which in itself is far from convincing. An increased concentration of lipoids in the "nutritive lymph stream" seems insufficient to account for the precipitation of lipoids in certain localized areas which show no normal differences from other comparable areas where lipoid deposits do not occur. For these and other reasons I feel that the "infiltration" theory, as it stands, does not provide a completely satisfactory explanation of the development of experimental cholesterol arteriosclerosis. I agree that the lipoids which appear in the arterial walls accumulate in such large quantities that they could not arise from local destruction of tissue. Chemical analyses to verify this point are not available, but it is so obvious that it is accepted as a fact by almost every one. It is not doubted, then, that the lipoids come from elsewhere to be deposited in the intercellular ground substance of the walls of the vessels. However, the conditions which bring about the precipitation of extraneous lipoids in the walls of arteries are not by any means clear. It seems worth while, therefore, to reconsider this aspect of the pathogenesis of experimental cholesterol arteriosclerosis.

If the lipoids which accumulate in the arterial walls come from elsewhere, they must be brought by way of the blood stream. From the experiments with colloidal dyes already mentioned, it seems clear that colloid-containing fluid derived from the blood plasma normally permeates the tissues of the wall of the vessel, partly from the lumen of the vessel and partly from the vasa vasorum. This fluid which serves the nutritional requirements of the tissues of the artery must be the immediate source of the lipoids which accumulate in the wall of the vessel. However, cholesterol and other lipoids are normally present in the rabbit's plasma, and presumably the nutritive tissue fluid contains a proportional amount of lipoids, but lipoid deposits do not occur in the arteries of rabbits fed on normal diets, not even when local injuries to the wall of a vessel are produced which are known to favor the deposition of lipoids. It follows, therefore, that some change in the character of the nutritive fluid which permeates the wall of the vessel must occur before the deposition of lipoids becomes possible.

Information as to the nature of this change in the character of the nutritive fluid should be forthcoming from the data concerning the

development of experimental cholesterol arteriosclerosis. Cholesterol feeding is the one factor which is known definitely to be essential to the production of the lipid deposits in the arteries. The consequences of cholesterol feeding therefore, merit first consideration. Following the ingestion of cholesterol, hypercholesteremia appears. But it is not so much dependent on an increase of free cholesterol in the blood as on an elevation of the esters of cholesterol with fatty acids, and, more than a simple hypercholesteremia, there is a general hyperlipemia. With these facts in view, there seems to be no good reason for focusing attention on the hypercholesteremia alone, especially since it is well known that the stability of cholesterol in solution depends to a great extent on the concentration of the other lipoids which are associated with it. However, almost all of the available data are concerned only with the cholesterol content of the blood so that one is forced to refer repeatedly to hypercholesteremia as though it were the only change in the blood which is worthy of consideration. Lack of more complete information is the only reason for doing so.

The assumption has already been made that some degree of hypercholesteremia is necessary to the development of experimental cholesterol arteriosclerosis in rabbits (section VI b). It is not known that this is the only necessary change in the blood, but granting that hypercholesteremia is essential, a variety of ways in which it could exert an influence may be imagined. One almost certain consequence of hypercholesteremia, and of the hyperlipemia which probably always accompanies it, is an increase in the lipid content of the nutritive fluid which permeates the walls of vessels. The nutritive fluid may be altered in other ways as well, but nothing has been sought or studied in the blood except the lipoids, so that an increased concentration of lipoids in the nutritive fluid is the only specific alteration which can be assumed with confidence. For the present, then, one must be satisfied with this as fulfilling the theoretical requirement that a change in the character of the fluid which permeates the arterial walls must occur before the deposition of lipoids become possible. In any event, it is obvious that following the feeding of sufficient quantities of cholesterol, the general conditions in the blood and secondarily in the nutritive fluid of the walls of vessels are suitable for the formation of lipid deposits in the walls of the arteries.

Now, since the lesions of experimental cholesterol arteriosclerosis are local and not diffuse, it is self-evident that the conditions in the wall of the artery at the sites where lipoids are deposited are different from those which obtain in the parts of the same artery where no lipid deposits occur. The conditions which exist at the sites of lipid deposi-

tion and which do not exist elsewhere must constitute the immediate cause of the precipitation of lipoids in those areas, and it is equally obvious that these conditions must exist before any precipitation takes place. It is important to inquire into the nature of these local conditions in the arterial wall which are responsible for the precipitation of lipoids from the colloidal state in which they enter the wall of the vessel with the nutritive fluid. For the sake of convenience these conditions will be referred to as the "local precipitating conditions"

It is conceivable that the local precipitating conditions exist normally in normal arteries, but this seems most improbable. The idea that the permeability of the endothelial lining of the aorta is normally greater in certain areas than in others is based on what seems to be a misinterpretation of the results of experiments with colloidal dyes. Careful repetition of these experiments⁴⁴ indicates that the lining of the aorta, as might be expected, is everywhere equally permeable to the dye. Indeed, it is difficult to imagine the existence of local precipitating conditions in normal arteries, especially since these conditions must exist only in certain local areas and not in others. In the aorta, for example, one would have to assume that the local precipitating conditions were present normally in certain areas, while in adjacent comparable areas they were not. If only the regions around the mouths of branching arteries became the sites of lipid deposits, one could believe that the unique conditions existing there might be sufficient to determine the deposition of lipoids in those areas and not elsewhere, but actually, localized lipid deposits can develop almost anywhere in the aorta and the pattern of distribution of the lesions is never twice exactly the same. In fact, the progressive appearance of increasing numbers of localized lesions in the arteries during the course of cholesterol feeding experiments gives clear indication that the local precipitating conditions in the walls of the vessels develop progressively after the experiment has been commenced. The appearance of the first patch of lipid deposit shows that the general conditions in the blood, etc., are suitable for the precipitation of lipoids in the arterial walls, and yet the other localized fatty lesions which develop subsequently make their appearance one by one as the experiment continues, deposition of lipoids commencing in each new area only after the appropriate local precipitating conditions have been established.

These partly theoretical considerations are verified by the actual observation of histologic changes in the arterial walls which develop during the course of cholesterol feeding experiments and which evidently precede the deposition of lipoids. In the intima, the ground substance becomes swollen so that the subendothelial layer is distinctly thicker than usual. The preliminary changes in the media are more conspicuous, consisting of focal necrosis and dissolution of muscle fibers

so that only a rather cloudy or flocculent ground substance remains. In both the intima and the media the altered ground substance subsequently becomes impregnated with stainable lipid particles. The preliminary histologic changes are apparently the visible expression of the development of the local conditions in the arterial wall which constitute the immediate cause of the precipitation of lipoids. It is impossible to say what invisible changes in conditions may accompany the visible alterations and consequently the exact manner in which the precipitation of lipoids is brought about cannot be specified. It is evident that the intercellular ground substance is altered in some way. Probably there is a change in its physicochemical state, a change perhaps akin to coagulation. Whether this alteration in itself is sufficient to explain the results is not known, it seems that it may well be. In any case, the sum total of the local change in conditions confers on the altered ground substance the property of accumulating precipitated lipoids from the nutritive fluid which permeates the walls of the vessels.

It seems evident that the preliminary local changes in the arterial walls are due to some kind of injury. This is especially obvious in the initial alterations in the media where there is actual necrosis of muscle fibers. It can be inferred from this that the associated changes in the intima are likewise due to injury. This conclusion receives the strongest sort of support from the evidence advanced in section VI *f* of this paper, and it is entirely consistent with every known fact concerning experimental cholesterol arteriosclerosis in rabbits. However, it is impossible at present to specify the cause of the arterial injury. This should be in no way surprising since the rôle of the primary injury to the arteries has never been emphasized previously, and the problem of its cause remains practically untouched. Evidently some unsuspected factor connected with the feeding of cholesterol is injurious to the rabbit's arteries, which are well known to be extremely susceptible to noxious agents of many kinds. I have pointed out that the preparations of supposedly pure cholesterol which have been used in the past were probably not composed of a single sterol¹¹⁹ but almost certainly contained ergosterol and possibly other sterols, which might well have a damaging effect on the arteries of rabbits even though present in the diet in relatively small quantities. Perhaps the feeding of large amounts of cholesterol and oil entails some other departure from a normal adequate diet. Possibly the hyperlipemia, which is often excessive, upsets the general metabolism sufficiently to effect an injury to the arteries. Danisch³⁷ suggested that nervous influences are of importance, but the cause of the arterial injury is really quite unknown and urgently requires investigation. However, this problem being left unsolved for the present, the fact remains that a primary injury to the arterial walls occurs and plays an essential part in the development of experimental cholesterol arteriosclerosis.

From all that has been said, it seems clear that the occurrence of localized lipid deposits in the walls of arteries is dependent on the coexistence of two sets of conditions. One set includes the conditions which must obtain in the blood and secondarily in the nutritive fluid which permeates the walls of the vessels. These may be called the general conditions. The second set comprises those conditions which must exist locally in the walls of the arteries at the sites where lipid deposits are to occur. These may be called the local conditions. When both the local and the general conditions are suitable, lipid deposits will develop. It seems probable that neither set of conditions exists normally in the rabbit. It has been assumed that hypercholesteremia with a consequent increase in the lipid content of the nutritive fluid is one of the necessary general conditions. In any event, no other general conditions have been recognized. The appropriate local conditions follow as the result of some sort of injury to the arterial walls. An alteration in the state of the intercellular ground substance of the vessel wall, due to the arterial injury, can be regarded as one of the necessary local conditions. No other local conditions have been recognized specifically.

Since both of the recognized factors—hypercholesteremia and injury to the arteries—are conceived as being necessary to the development of experimental cholesterol arteriosclerosis in rabbits, it follows that either factor existing alone will be ineffective in producing the fatty lesions in the arteries. It is well known that the lesions produced in the arteries of rabbits by various kinds of injury (e.g., cauterization, injections of epinephrine or nicotine, etc.) always remain practically free from stainable fat so long as the blood lipoids are at normal levels. It is also clear that hypercholesteremia of marked degree can follow cholesterol feeding in rabbits without producing any lesions in the arteries. This fact has been pointed out already in section VI *b* of this paper. Moreover, it has been shown in section VI *f* that the general conditions in the blood may be suitable for the development of lipid deposits in the arterial walls without the occurrence of any such deposits in normal parts of the arteries. In the same place it was pointed out that the most abundant accumulations of lipoids develop when the feeding of cholesterol is supplemented by other procedures which injure the arteries more rapidly and severely. All of these facts confirm the general conceptions set forth in the preceding paragraph.

When the precipitation of lipoids in the arterial walls has been accounted for, the further development of the lesions of experimental cholesterol arteriosclerosis follows naturally enough. The increasing abundance of the lipoids in the arterial lesions evidently depends on the continued action of the factors which are responsible for the initiation of lipid deposition. Evidence of the continuation of injury to the arteries can be found in the progressive destruction of muscle and

elastic tissue in the inner layers of the media. In the intima, however, all else is masked by the density of the lipoid deposits and by the cellular proliferation which follows. The proliferative response is generally regarded as a direct result of the presence of precipitated lipoids in the intima, special emphasis being laid on the presence of cholesterol esters. Such a relationship is obvious in the case of the macrophages which appear in the intima and take up the lipoids with such avidity. It seems probable, too, that the presence of lipoids can stimulate the proliferation of fibrous connective tissue cells, but the effect of the primary injury in producing a reparative reaction cannot be disregarded. Nevertheless, all that can be said is that the local injury to the wall of the vessel which leads to the precipitation of lipoids, together with the presence of the lipoids themselves, results in a proliferation of fibrous connective tissue cells in the intima. With the increase in thickness of the intima and with the appearance of necrosis in its deeper layers, the whole process becomes so complicated that it is impossible to analyze with any chance of accuracy the rôle of individual factors.

In accordance with the discussion presented in the foregoing pages, the pathogenesis of experimental cholesterol arteriosclerosis in rabbits may be summarized as follows. Cholesterol feeding produces general alterations in the blood and secondarily in the nutritive fluid which permeates the walls of the arteries. Among these changes, hypercholesteremia and an increased concentration of lipoids in the nutritive fluid are the only ones which have been recognized up to the present time as being of importance in the formation of lipoid deposits in the arterial walls. In addition to these general changes, some unknown factor inherent in the experimental procedure of cholesterol feeding produces an injury to the walls of the arteries. This injury is responsible for the local histologic changes in the walls of the vessels which precede the appearance of lipoid deposits. In the presence of the appropriate general conditions in the blood and nutritive fluid, lipoids are precipitated from the latter in the injured areas of the arterial walls through the influence of the special local conditions which exist in such areas. The existence of these local conditions is essential to the precipitation of lipoids, and consequently lipoid deposits do not develop in the remaining normal parts of the arteries. The subsequent development of the arterial lesions depends on the continued action of the factors which are responsible for their initiation. However, the presence of precipitated lipoids in the arterial walls constitutes another important local factor, which, together with the original local injury, leads to the subsequent cellular proliferation in the intima. Beyond this point, the development of experimental cholesterol arteriosclerosis is not capable of accurate analysis.

IX ANATOMIC COMPARISON OF EXPERIMENTAL CHOLESTEROL
ARTERIOSCLEROSIS AND HUMAN ARTERIOSCLEROSIS

From the description of the lesions of experimental cholesterol arteriosclerosis in rabbits which has been given in section IV of this paper, it is obvious that there are certain points of resemblance between them and the lesions of arteriosclerosis in man, but it is equally obvious that there are a number of differences between the two. The gross appearance of the individual lesions in the arteries of the experimental animal is not unlike that of certain lesions of human arteriosclerosis and in some respects their distribution is similar. The experimental arterial lesions frequently involve areas about the mouths of branching arteries, in much the same way as in human arteriosclerosis. Amitschkow¹² and others have pointed out that the localization of the early lesions of experimental cholesterol arteriosclerosis in the aorta shows the closest correspondence with the distribution of the minute lipid flecks found in the aortas of children, but whether or not the latter represent the initial stage of human arteriosclerosis is, of course, another question. On the other hand, the lesions in the rabbit's aorta tend to become most advanced in the arch, while in man it is a common observation that arteriosclerosis usually reaches its greatest severity in the abdominal portion of the aorta. In the rabbit, the pulmonary artery and its branches are affected to a degree proportional to the severity of the lesions in the aorta, while in man it is well known that the pulmonary artery is usually spared even in the presence of advanced arteriosclerosis in the aorta and elsewhere. In experimental cholesterol arteriosclerosis, the renal arteries are affected rather infrequently, but perhaps the most striking divergence from the distribution of arteriosclerosis in man is to be found in the fact that the cerebral and retinal arteries are never involved in experimental cholesterol arteriosclerosis.

Microscopically, no comparison can be drawn between the very early stages of the arterial lesions in the rabbit and in the human being for the reason that in the latter there is still no general agreement as to what may constitute the earliest change. However, the slightly more advanced lesions of experimental cholesterol arteriosclerosis exhibit a number of points of similarity to certain lesions of human arteriosclerosis. There is a localized thickening of the intima in which doubly refractive and other lipoids are present in extraordinary quantities. Large fat-containing macrophages or foam cells are present in abundance, and there is some proliferation of fibrous connective tissue cells. The lipoids are found within cells and densely strewn through the intercellular ground substance, although they are not concentrated along elastic fibrils in the intima as they so frequently are in human arteries. Among the lipoids, cholesterol and its esters seem to be present in greatest abundance as in

human arteriosclerosis^d An even closer resemblance to arteriosclerosis in man is found in the more mature arterial lesions which develop in rabbits after a long period of cholesterol feeding followed by a longer period during which cholesterol is withheld from the diet In such lesions, lipoids are not so abundant, foam cells are present in much smaller numbers, and the intimal thickening is predominantly fibrous Altogether, then, the lesions in the arteries, so far as the changes in the intima are concerned, simulate very closely the lesions of human arteriosclerosis However, the same statement cannot be made when the lesions in the media are taken into consideration

In rabbits, following cholesterol feeding anisotropic lipid deposits sometimes appear in the inner layers of the media before there is any visible change in the overlying intima, or they may appear at a very early stage in the development of the intimal lesions Lipoid deposits in the media have never been described as a feature of the early stages of the development of arteriosclerosis in man When lipid deposits in the media occur in human arteriosclerosis, they are found beneath well developed intimal plaques Moreover, the lipoids appear first between the muscle fibers and the adjacent elastic laminae, both of which remain intact for a considerable time afterward In the rabbit, on the other hand, the appearance of the anisotropic lipoids in the media follows destruction and disappearance of muscle and elastic tissue So far as the histologic changes in the media are concerned, therefore, the arterial lesions of experimental cholesterol arteriosclerosis do not correspond at all closely with those of arteriosclerosis as it occurs in man This fact may account for the apparent reluctance of some investigators to describe specifically the condition of the media in experimental cholesterol arteriosclerosis, and may explain their preference for noncommittal descriptions of the arterial lesions in which repeated references are made, not to the intima or media, but to "the inner layers of the arterial wall"

In addition to the lesions in the arteries, the associated changes in other tissues which occur during the development of experimental cholesterol arteriosclerosis must be taken into consideration It has been mentioned already that in the usual course of cholesterol feeding in the rabbit there is brought about an accumulation of anisotropic lipoids in various situations other than the walls of arteries^e Lipoids accumulate in the cells of the suprarenal cortex, in the liver cells and in the reticulo-endothelial cells of the liver, spleen, lymph nodes and bone marrow These lipid accumulations appear before any changes are to be found

(d) 136, 175, 182 See also the chemical analyses of the lipoids contained in arteriosclerotic lesions of the human aorta reported recently by Meeker and Jobling (*Arch Path* 18 252, 1934)

(e) 34, 135, 165, 189

in the arteries, and they persist as long as cholesterol feeding is continued. This phenomenon has no counterpart in arteriosclerosis in man. There are those who claim that there is an unusual abundance of lipoids in the suprarenal cortex in association with human arteriosclerosis, but this is open to doubt and certainly has never been satisfactorily established. On the other hand, it is admitted by all that no abnormal accumulation of doubly refractive lipoids in the cells of the reticulo-endothelial system or in the liver cells occurs in association with human arteriosclerosis. Attempts have been made to minimize this discrepancy on the ground that experiments can be so arranged as to avoid the accumulation of such excessive amounts of lipoids in the reticulo-endothelial cells during the development of lesions in the arteries. This can be accomplished by feeding very small quantities of cholesterol over a period of several years, but even under these conditions there are found relatively slight but definitely abnormal lipoid accumulations in the cells of the reticulo-endothelial system.⁷ Lipoids may disappear almost completely from the reticulo-endothelial cells if cholesterol feeding is discontinued after arterial lesions have been produced, and if a long period of time is then allowed to elapse before the experiment is terminated. However, one can feel perfectly certain that the reticulo-endothelial cells were laden with anisotropic lipoids during the period when the arterial lesions were developing, a condition which is not observed as an accompaniment of the development of arteriosclerosis in man.

The lipoid deposits which may appear in the rabbit's cornea as a result of cholesterol feeding have been described by Verse¹⁶³ and others as the analog of the arcus senilis in man. However, the arcus senilis does not occur with great regularity in association with human arteriosclerosis, as the name implies, it is generally associated with old age rather than with arteriosclerosis as such. Accumulations of anisotropic lipoids in the interstitial tissue of the kidneys occur in connection with experimental cholesterol arteriosclerosis. These lesions, which have been described in detail by Bailey²⁰ and Schonheimer,¹³⁵ develop in the absence of any changes in the renal arteries which, as has been pointed out, are seldom affected in the experimental disease. It is evident that no similar lesions in the kidneys are associated with arteriosclerosis in man. Deposits of doubly refractive lipoids have been described as occurring in various other tissues of rabbits in which experimental cholesterol arteriosclerosis has been produced. Such deposits have been found in the skin, subcutaneous tissue and tendons and in the mucosa and submucosa of the gallbladder, bile ducts, stomach and intestine.^f Although careful search, perhaps, has not been made in human cases, similar accumulations of anisotropic lipoids have never been described as a regular accompaniment of arteriosclerosis in man.

(f) 34, 135, 165, 189

The obvious purpose of drawing a comparison between the anatomic lesions of experimental cholesterol arteriosclerosis and those of human arteriosclerosis is to determine whether or not the experimental disease can be considered from the anatomic point of view as a reproduction of arteriosclerosis as it occurs in human beings. Enough has been said already to show that the arteriosclerosis produced experimentally in rabbits by cholesterol feeding is not anatomically identical with human arteriosclerosis. The similarity between the arterial lesions in the two instances is very striking and there need be no hesitation in stating that experimental cholesterol arteriosclerosis resembles arteriosclerosis in man more closely than any experimental lesions of arteries which have yet been produced by procedures other than cholesterol feeding. Nevertheless, the differences must be taken into account.

It is contended by some that the explanation of these differences lies in the normal anatomic differences between the rabbit and man, such, for example, as exists in the structure of the intima of the aorta. But who can say how much should be allowed for such differences in anatomic structure? It is conceivable that the differences between the arterial lesions in the two instances may be explained on this basis. However, one can say only that this explanation is possibly correct or perhaps correct in part, but not necessarily so. The accumulations of anisotropic lipoids in various situations other than the arteries in experimental cholesterol arteriosclerosis, which do not occur in connection with human arteriosclerosis, seem inexplicable on the grounds of anatomic differences. Lesions similar to those observed in cholesterol-fed rabbits (e. g. xanthomas of the skin and internal parts and overloading of the reticulo-endothelial system with various lipoids) are encountered on occasion in the field of human pathology,⁴ it is clear that such lesions can develop in the human body, but they do not occur in any close or constant association with arteriosclerosis. The close association of extra-arterial lipid accumulations with arteriosclerosis is a feature peculiar to the experimental disease.

Altogether, it seems that the anatomic picture of experimental cholesterol arteriosclerosis in rabbits shows predominantly a saturation of the whole body with lipoids. These accumulate in excessive quantities in the cells of the liver, suprarenal cortex and reticulo-endothelial system and, in addition, they are precipitated in the intercellular substance of various tissues wherever the local conditions are suitable, whether in the arteries or elsewhere. On the other hand, the whole anatomic picture of human arteriosclerosis exhibits a process in which the rôle of the lipoids is confined almost exclusively to their much less conspicuous accumulation in the arterial lesions. All of this suggests very strongly that the part played by the lipoids in the development of arteriosclerosis,

whatever that part may be, is greatly exaggerated in experimental cholesterol arteriosclerosis as compared with arteriosclerosis in man. Recognition of this general difference is of essential importance to a correct interpretation of the significance of the experimental data.

The only definite conclusion which can be drawn from an anatomic comparison of experimental cholesterol arteriosclerosis with human arteriosclerosis is that the two are not completely identical. An exact evaluation of the importance of the differences will not be attempted, on this point there can be no final judgment at present. Certainly one is not justified in taking such an insecure position as that of Anitschkow, who sweeps aside all differences with the mere statement that "none of these differences is of an essential nature"¹². One can proceed further only on the ground that the anatomic similarity between experimental cholesterol arteriosclerosis and human arteriosclerosis should permit one to expect some features of similarity in their etiology and pathogenesis. Nevertheless, the anatomic differences between the two should be sufficient caution against the hasty conclusion that their etiology and pathogenesis are of necessity exactly or even nearly identical, or that the etiologic factors which may be common to the experimental and human diseases must operate with equal intensity in both instances.

X SIGNIFICANCE OF EXPERIMENTAL DATA IN RELATION TO ETIOLOGY AND PATHOGENESIS OF HUMAN ARTERIOSCLEROSIS

Various factors of possible importance in the etiology of experimental cholesterol arteriosclerosis have been considered individually in section VI of this paper, and the pathogenesis of experimental cholesterol arteriosclerosis in rabbits has been discussed in section VIII. On the basis of the available experimental evidence brought forward in those discussions, three main factors were implicated in the production of the lesions in the arteries of rabbits, namely, the presence of considerable quantities of cholesterol in the diet, the existence of hypercholesteremia and the occurrence of injury to the arteries. To assume that these same three factors are all in operation in the development of human arteriosclerosis, or that they operate in man in exactly the same way as in the rabbit, is entirely without justification, not only for the reasons pointed out in the foregoing section (section IX) but also because there are certain differences between the rabbit and man which have not yet been touched on and which place further obstacles in the path of any attempt to make a direct application of the inferences drawn from the experimental data.

Some differences between the rabbit and man which are of obvious importance in this connection may be pointed out briefly. Among these is the fact that the rabbit is a herbivorous animal while man is omnivorous. Cholesterol is completely lacking in vegetable diets, although

phytosterol, the plant relative of cholesterol, is present in comparatively small amounts. On the other hand, cholesterol is present in varying quantities in the normal diets of omnivorous animals. Perhaps related to this difference in normal diet is the extreme tardiness with which the rabbit excretes exogenous cholesterol as compared with the rapid excretion of cholesterol in man.¹¹ This deficiency of the rabbit's excretory apparatus serves to explain the ease with which hypercholesteremia and hyperlipemia can be produced in rabbits by means of cholesterol feeding. In man, on the contrary, large quantities of cholesterol in the diet have only a relatively slight and temporary effect on the level of cholesterol in the blood,¹ owing apparently to the facility with which cholesterol is disposed of by the human mechanism. Another difference of importance is that which exists between the normal cholesterol content of the blood of the rabbit and that of man. As may be seen from the data collected by Weidman and Sunderman,¹⁷⁰ the normal range of the blood cholesterol in rabbits, as determined by various investigators, is far below that found in man by corresponding methods of cholesterol estimation. Taking the median point of the normal range in each case, the cholesterol content of human blood is approximately twice as great as that of the rabbit's blood. The last difference which need be mentioned is of no less importance than the others. The typical spontaneous arteriosclerosis of rabbits shows a lack of any lipid deposits in the arterial lesions. Using the word "spontaneous" in the same sense, the typical spontaneous arteriosclerosis of man is characterized almost constantly by the presence of more or less extensive accumulations of lipoids in the affected parts of the arteries. One can hardly avoid the suspicion that this divergence between the respective types of spontaneous arteriosclerosis may be related to the difference between the normal lipid content of the blood of the rabbit and that of the human being.

These differences between the rabbit and man complicate the interpretation of the experimental results and render it impossible to evaluate the significance of the experimental data without making careful allowances for the differences which have been mentioned. Such being the case, it is rather surprising that any one should consider it fortunate that the rabbit is the animal in which the cholesterol feeding experiments have proved successful. Nevertheless, Anitschkow¹² has contended that the rabbit is an especially suitable animal for experimental investigations on arteriosclerosis simply because it is not subject to the spontaneous development of arterial lesions resembling those of human arteriosclerosis. Actually, this is one of the best reasons for subjecting to the closest possible scrutiny the inferences drawn from the experimental data obtained in rabbits.

(h) 28, 135

(i) 28, 52, 107, 144

In order to produce arterial lesions resembling those of human arteriosclerosis in an animal in which the spontaneous arteriosclerosis of "old age" is of a quite different type, one must really do two distinct things. First of all, the animal must be converted into one which is susceptible to the development of arterial lesions of a character quite foreign to it. In this artificially prepared animal, a second factor or group of factors must then be introduced for the actual production of the lesions in the arteries. Obviously, the second step is the only one that could be of any importance in relation to human arteriosclerosis. But it happens that all of this can be accomplished in the rabbit by the seemingly simple procedure of feeding large quantities of cholesterol. Now, under these conditions, it is difficult to determine from the experimental data alone what it is that converts the rabbit into a susceptible animal and what it is that is responsible for the actual production of the characteristic lesions in the arteries. On the basis of the evidence brought forward in this paper, it seems most likely that the rabbit is rendered susceptible to the development of arterial lesions simulating those of human arteriosclerosis by the elevation of its blood cholesterol level, which is normally much lower than that in man and which is raised by cholesterol feeding. The actual cause of the lesions in the arteries is an injury to the arterial walls which is produced by feeding large quantities of cholesterol or cholesterol-rich foods, but which is not caused directly by the resulting hypercholesteremia. If all of this is true, as is probably the case, it follows that the hypercholesteremia as the preparatory factor has no corresponding importance in relation to human arteriosclerosis, but the injury as the exciting factor must be of the greatest significance. As I shall show presently, the available evidence derived from the study of human material corresponds precisely with this idea.

The major difficulties in estimating the significance of experimental arteriosclerosis produced in animals would be removed by selecting for experimentation an animal species which is closely related to man and which is naturally subject to the development of a type of spontaneous "old age" arteriosclerosis which resembles human arteriosclerosis as closely as possible. The obvious aim, then, would be to produce prematurely in such an animal lesions of the arteries of a character corresponding with its own type of spontaneous arteriosclerosis. Of course, the possible spontaneity of the lesions supposedly produced by the experimental procedure would have to be excluded by the examination of adequate numbers of control animals. So far as I am aware, successful experiments of this nature have never been reported. However, if positive experimental results could be obtained under the conditions described, the significance of those results would not remain long in doubt.

Returning to the cholesterol feeding experiments in rabbits, I wish to reemphasize the fact that the differences between the experimental animal and man, added to the anatomic differences between experimental cholesterol arteriosclerosis and human arteriosclerosis, render the problem of interpretation far from simple. In the past, the difficulties of interpretation have never been given adequate consideration, and inferences regarding human arteriosclerosis have been drawn with the greatest abandon from the experimental data. A much too enthusiastic and zealous faith in the validity of the experimental results has led to the blind acceptance of conclusions which have been quite misleading. The outstanding example of this free exercise of faith is the general belief that hypercholesteremia is a factor of paramount importance in the etiology of arteriosclerosis in man, a belief which is based entirely on the probability that hypercholesteremia is essential to the development of experimental cholesterol arteriosclerosis in rabbits.

Anitschkow³ has adroitly turned to advantage the difficulties of interpretation which he is willing to recognize as such. He has expressed the belief that the unique experimental procedure, the relatively short duration of the experiments and the differences between the rabbit and man are just sufficient to explain the differences between the total picture of experimental cholesterol arteriosclerosis and that of human arteriosclerosis. Having offset all of these differences, one against another, he has proceeded with the apparent conviction that the etiology and pathogenesis of experimental cholesterol arteriosclerosis and of human arteriosclerosis are essentially the same. Such an assumption is not justified by the known facts. Aschoff,¹⁷ on the other hand, pointing especially to the difference in cholesterol excretion between the rabbit and man, has made the following comment: "For this reason one should not attempt to apply the conclusions of experiments with rabbits too freely, and their interpretation in human beings must be accepted with reserve." This attitude of caution seems imperative if gross misinterpretations are to be avoided.

When all of the circumstances are taken into consideration, it becomes perfectly obvious that the application of conclusions drawn from the experiments cannot be justified without some evidence of the existence of comparable conditions in the human being. A proper appreciation of the significance of the experimental results can be gained only through correlation of the experimental data with corresponding data derived from the study of human material. Accordingly, it will be necessary to consider individually the principal factors involved in the development of experimental cholesterol arteriosclerosis and to

determine whether or not there is any indication of the operation of similar factors in the development of human arteriosclerosis

(a) *Cholesterol in the Diet*—In section VI of this paper, reasons have been given for the conclusion that the presence of considerable quantities of cholesterol in the rabbit's diet is an essential factor in the production of experimental cholesterol arteriosclerosis. In the same section it was shown that one important result of the administration of cholesterol in the diet is hypercholesteremia, a condition which was assumed to be also an essential factor in the development of the arterial lesions in rabbits. Is there now any reason for believing that cholesterol in the diet of human beings plays a comparable rôle in the development of human arteriosclerosis? This question can be answered at once in the negative on the following grounds

In the human being, in contrast with the rabbit, cholesterol is a normal constituent of the diet, being contained in considerable quantities in eggs, milk, butter and meat. The amount of cholesterol ingested undoubtedly varies considerably between persons, and from time to time in any single person, but these variations in the cholesterol content of the diet have never been shown to have any significant effect on the cholesterol content of the blood. Furthermore, the deliberate addition of cholesterol to the food of normal subjects does not produce a sustained hypercholesteremia, the effect on the level of cholesterol in the blood is relatively slight and transitory.¹

Now, in spite of the differences in the cholesterol content of the diets of different persons and of the various diets of different races, it has never been shown that those persons or races which subsist on diets poor in cholesterol are by any means protected against the development of arteriosclerosis. Nor has it been shown that those persons or races which develop arteriosclerosis at an earlier age or in more severe degree than others are especially partial to foods which are rich in cholesterol. On this point one may quote some remarks of Weiss and Minot,¹⁷⁴ who have reviewed the literature bearing on nutrition in relation to arteriosclerosis: "It cannot be stated that overnutrition with lipoid and fatty substances plays a rôle in the production of arteriosclerosis in normal man. Statements that fat is responsible for arteriosclerosis are not lacking in the literature, but these statements are not based on controlled observations, and they seem to be strongly influenced by the imbibition theory of arteriosclerosis proposed by Virchow and amplified recently by Aschoff and Anitschkow. Caution is necessary at this point lest we fall into the so often dangerously applied *post hoc propter hoc* method of reasoning." As Weiss and Minot have pointed out, all of the evidence which has been brought forward in the attempt to demonstrate a relationship between

(1) 28, 52, 107, 144

the cholesterol content of the diet and the development of arteriosclerosis in man is equivocal, and therefore, far from convincing. Indeed, it would hardly influence an opinion not already prejudiced in favor of the idea.¹

In considering the significance of the experimental data, the results of cholesterol feeding experiments in animals other than the rabbit cannot be overlooked. It is especially important to bear in mind that animals such as cats and dogs which, like man, fail to show marked hypercholesteremia following cholesterol feeding, do not show any changes in their arteries after prolonged periods of feeding on diets rich in cholesterol. The uniform failure of all attempts to produce arterial lesions by this method in cats, dogs, foxes and monkeys speaks strongly against the idea that cholesterol in the diet is a factor of any importance in the etiology of human arteriosclerosis. The negative results obtained in these animals seem more properly applicable to the human being than do the positive results obtained in rabbits.

The considerations set forth in the preceding paragraphs lead to the conclusion that the results of cholesterol feeding experiments in rabbits do not constitute a valid reason for believing that an excess of cholesterol in the diet plays a rôle in the etiology of arteriosclerosis in man. No convincing evidence in support of such a belief exists at present.

(b) *Hypercholesteremia*—In section VI (b) of this paper it was pointed out that a well marked or even extreme hypercholesteremia, and indeed a general hyperlipemia, almost constantly precedes and accompanies the development of experimental cholesterol arteriosclerosis. In the same place, the tentative conclusion was reached that some elevation of the blood cholesterol is necessary to the development of the fatty arterial lesions in rabbits. The probability that this is true is supported by certain theoretical considerations brought forward in section VIII, where it was also concluded that hypercholesteremia is effective only in the presence of local injury to the walls of the arteries. These conclusions, of course, applied only to the development of experimental cholesterol arteriosclerosis in rabbits. In man, on the contrary, no analogous changes in the blood are found to occur in connection with arteriosclerosis. No change in the blood lipoids has been shown to exist as a sign of the impending development of arteriosclerosis in man, nor has any change in the lipid content of the blood been found as a constant accompaniment of any stage in the progress of the disease.

It is sometimes stated that hypercholesteremia is regularly associated with arteriosclerosis in man, but such statements are based on a litera-

(1) The status of this question is not altered by the recent publication of Rosenthal (Arch Path 18 473, 1934). The evidence which he has advanced is of the same character as that adduced in the previous literature and is open to the same objections.

ture much of which has no direct bearing on the question. Some publications which are frequently referred to in this connection have nothing to do with arteriosclerosis as such, but are concerned with various other diseases in which hypercholesteremia is known to occur and with which arteriosclerosis may be associated. Other reports deal with the occurrence of hypercholesteremia in association with high blood pressure. In some of these publications, cases of "essential" hypertension have been selected for study, but in others the clinical cases have been chosen with high blood pressure as the only qualification, so that diseases of the greatest variety have been included (even diabetes mellitus!). These studies of hypertension, whether in carefully selected cases or otherwise, have yielded the most conflicting results. Some investigators have found hypercholesteremia in a proportion of their cases varying from 13 to 76 per cent,^m while others have found normal blood cholesterol levels in practically all cases of their series.ⁿ Some of these authors believe that hypercholesteremia causes hypertension, some believe that hypertension causes hypercholesteremia, others contend that the two are not related. Whatever may be the truth of the matter, it is obvious that these data prove absolutely nothing regarding the occurrence of hypercholesteremia in association with anatomic lesions of the arteries.

The publications which contain direct information as to the occurrence of hypercholesteremia in association with human arteriosclerosis are relatively few. Weltmann,¹⁷⁶ without giving data on individual patients, stated that in general he had observed increased blood cholesterol in his arteriosclerotic patients, some of whom had apparently shown hypertension. Koulikov and his collaborators⁷⁷ made determinations of the blood cholesterol in 50 arteriosclerotic patients and found values above 160 mg per hundred cubic centimeters in 20 cases. Mjassnikow¹⁰² observed elevation of the blood cholesterol level in 30 cases of arteriosclerosis among the 70 which he studied. He found hypercholesteremia most consistently in patients suffering from far advanced arteriosclerosis of the aorta and coronary arteries, while it occurred with rapidly diminishing frequency in the less severe cases. On the other hand, Stepp¹⁵⁰ could find no abnormality in the levels of cholesterol in the blood in cases of arteriosclerosis without hypertension. In the cases of arteriosclerosis studied by Denis,³⁹ the cholesterol content of the blood was normal regardless of the presence or absence of hypertension.

From all of this, one can conclude only that hypercholesteremia may be found in certain cases of advanced arteriosclerosis and not in others. All of these cases are properly described as "advanced," for

(m) 3, 54, 122, 168, 173, 181

(n) 58, 91, 102

in all instances the diagnosis of arteriosclerosis was made from clinical study alone, and it is obvious that arteriosclerosis of sufficient degree to be recognized clinically is far past the initial stages. Now, if, as Mjassnikow^{10,2} claimed the incidence of hypercholesteremia is highest among those patients with the most extreme arteriosclerosis and lowest among those least affected, the occurrence of hypercholesteremia might more logically be regarded as the effect rather than the cause of arteriosclerotic changes which have already developed. Bruger and Poin-dexter,⁷ in a recent study of the plasma cholesterol in relation to the development of arteriosclerosis in obese persons, reached practically the same conclusion. However, one need not dwell on this point, for the available data, including the results of those who seem most eager to demonstrate an elevation of the lipoids in the blood, fail to give convincing evidence that hypercholesteremia occurs in anything approaching a close association with arteriosclerosis. On this question, then, a final conclusion may be expressed in agreement with such disinterested authorities as Buiger,²⁵ Gardner²¹ and Peters and Van Slyke,¹¹⁰ who are satisfied to dismiss the subject with the simple but significant statement that hypercholesteremia is not found with any regularity in association with arteriosclerosis in man.

The fact that an elevation of the cholesterol or of other lipoids in the blood is not found as an accompaniment of arteriosclerosis in man constitutes a strong argument against the idea that such a disturbance of the blood lipoids is the cause of arteriosclerosis or that it is an essential factor in the development of the arterial lesions. Indeed, if hypercholesteremia were the cause of arteriosclerosis or an indispensable factor in the etiology of the disease, it would necessarily precede the development of lesions in the arteries but this has never been shown to occur, nor is it even suggested by any recorded observations. Actually, the only reason for suspecting hypercholesteremia as a factor in the etiology of human arteriosclerosis lies in the results of the cholesterol feeding experiments in rabbits. While these results form adequate grounds for the suspicion, they do not and cannot form adequate grounds for the conclusion that hypercholesteremia is necessary to the development of arteriosclerosis in man. In view of the data derived from the study of human material, it seems highly probable that arteriosclerosis in man can and usually does develop without deviation of the cholesterol content of the blood beyond the normal limits of variation. In any event there is no valid evidence to support any other conclusion.

The apparent discrepancy between the two conclusions that hypercholesteremia is an indispensable factor in the etiology of experimental cholesterol arteriosclerosis in the rabbit and that it is not necessary to the development of arteriosclerosis in man, is not by any means inexpli-

cable As I have pointed out, the level of cholesterol in the blood is normally very low in rabbits, in human beings, on the other hand, it is normally much higher Not only is cholesterol present in greater concentration in human plasma, but it normally stands at a level not far from the point of saturation¹⁷⁵ Under these conditions, it is not surprising that a blood cholesterol content within the normal limits should suffice for the precipitation of cholesterol in the walls of human arteries wherever the local conditions are favorable for its accumulation In the rabbit, following cholesterol feeding, the content of cholesterol in the blood is increased greatly, often to an extreme degree This corresponds with another observation to which I have called attention, that the anatomic lesions of experimental cholesterol arteriosclerosis give clear indication of an abnormal saturation of the whole body with lipoids The abundance and widespread distribution of the lipid deposits are out of all proportion to anything of a similar nature that occurs in association with human arteriosclerosis

These facts provide a reasonable explanation for the phenomena actually observed in the rabbit, and in man Thus, in the rabbit, in which the normal level of cholesterol in the blood is low, there occurs a type of spontaneous arteriosclerosis the lesions of which are practically devoid of stainable fat In the cholesterol feeding experiments when the blood lipoids are elevated, cholesterol and other lipoids are deposited in abundance, not only in the lesions in the arteries but also in various other tissues throughout the body In man, on the contrary, the normal level of cholesterol in the blood is relatively high, so that the lesions which develop in the arteries may become impregnated with lipoids without any change in the concentration of cholesterol in the blood At the same time, since the blood cholesterol is not elevated, there is no accumulation of lipoids in the cells of the reticulo-endothelial system nor any abnormal lipid deposits in other tissues Of course, it is implied that lipoids are deposited in the walls of human arteries as in the arteries of rabbits, only in those areas in which the local conditions have been rendered suitable for the precipitation of lipoids by the prior occurrence of local alterations in the walls of the vessels, a proposition which I shall presently defend This tentative scheme of the relationship between experimental cholesterol arteriosclerosis and human arteriosclerosis brings into accord what might otherwise appear to be conflicting conceptions It is of greater importance that this end is accomplished without doing violence to the facts as they are known at present

In discussing the pathogenesis of experimental cholesterol arteriosclerosis in rabbits, it has been concluded that hypercholesteremia acting alone does not produce the experimental lesions of the arteries The lesions develop only when hypercholesteremia and local injuries to the

walls of the arteries are combined. The experiments therefore give no reason for believing that hypercholesteremia when it occurs in human beings can of itself produce arteriosclerosis. However, it might be supposed on the basis of the experimental evidence that hypercholesteremia in man would accelerate the development of whatever arteriosclerotic changes happened to be already in progress. This supposition seems reasonable enough, and yet actual observation of patients in whom hypercholesteremia is known to exist does not offer much support for the idea. At the same time, a study of such patients provides convincing evidence to show that hypercholesteremia of itself is not a cause of arteriosclerosis.

I am aware that the high incidence of arteriosclerosis among patients with diabetes mellitus is frequently cited as evidence of the ability of hypercholesteremia to produce arteriosclerosis in man. This is a disease in which hyperlipemia and hypercholesteremia are well known to occur, but it is hardly necessary to point out that the metabolic disturbance in diabetes is not by any means confined to a disturbance of lipid metabolism or to an elevation of the lipoids in the blood. The well recognized tendency of diabetic patients to have arteriosclerosis is not necessarily due to the existence of hypercholesteremia. Hyperglycemia, ketosis or the frequent occurrence of infections in association with diabetes could just as well be held responsible. Moreover, it is not at all clear that the severity of the arteriosclerotic changes runs parallel with the degree of elevation of the blood lipoids. With this point in mind, Hunt⁹³ followed the progress of a group of diabetic patients and found that she could not establish any direct relationship between the elevation of the blood cholesterol level and the degree of arteriosclerosis which developed. Indeed, in her series of cases, the most advanced arteriosclerotic changes appeared in those patients whose average blood cholesterol level had been lowest.

If hypercholesteremia in man really can produce arteriosclerosis or can exert an appreciable influence on its development, abundant opportunities to observe these effects are offered by the occurrence of hypercholesteremia in association with a variety of conditions other than diabetes mellitus, e g, pregnancy, hypothyroidism, obstructive jaundice, certain types of nephritis and lipoid nephrosis.⁹ In spite of these opportunities, it has never been shown that the degree of arteriosclerosis found at autopsy in patients with such conditions is appreciably greater than might be expected in any other subjects of the same age, certainly to ordinary observation no difference is apparent. This is especially striking in children who come to autopsy after suffering even for a period of several years from lipoid nephrosis with constant or intermittent hypercholesteremia of extreme degree. In these cases, there are

no apparent alterations in the arteries, or none which might not be found in children of the same age dying from any other disease²⁵ These negative observations in patients with outspoken hypercholesteremia of considerable duration constitute the strongest sort of evidence against the idea that hypercholesteremia acting alone can cause arteriosclerosis. There is every reason, then, to conclude, in agreement with Burger,²⁸ that hypercholesteremia of itself cannot be regarded as a cause of arteriosclerosis in man. Although hypercholesteremia, when it occurs, might be expected on theoretical grounds to accelerate the development of arteriosclerotic changes which have already been initiated, there is little evidence at present to indicate such an effect.

Now, in spite of the fact that the data derived from the study of human material offer no support whatever, Anitschkow^p has insisted that hypercholesteremia resulting from some disturbance of cholesterol metabolism is responsible for the development of arteriosclerosis in man, an idea based entirely on the results of cholesterol feeding experiments in rabbits. He has always avoided stating directly that hypercholesteremia acting alone can cause arteriosclerosis in human beings and has admitted that factors which produce local injury to the arterial walls must be of importance, but only of secondary importance. He has expressed the belief that hypercholesteremia of very slight degree may be sufficient to cause arteriosclerosis "provided only that it is of long duration and associated with other injurious factors."¹² One cannot deny the possibility that fluctuations of the blood cholesterol so slight as to escape detection may occur. But the chemical methods in general use are accurate enough to determine the normal fluctuations of the level of the cholesterol in the blood. It is probable, therefore, that a significant hypercholesteremia could also be detected, but it has not been found. Moreover, as I have shown, it is unnecessary to assume that even a slight degree of hypercholesteremia is essential to the development of human arteriosclerosis.

Anitschkow's further suggestion of a "primary" disturbance of cholesterol metabolism as the underlying cause of arteriosclerosis in man is, of course, merely an invention to provide some reason for the hypothetical elevation of cholesterol in the blood. This idea is not supported even by the experimental evidence, much less by observations on human beings. Nevertheless Anitschkow,¹² in speaking of the etiology of human arteriosclerosis, has made the following statement: "On the basis of all these experimental results, it may therefore be regarded as definitely established that cholesterol, or rather a disturbance of the cholesterol metabolism, is of decisive significance as far as the genesis of atherosclerosis is concerned." For the reasons that have been given, one cannot accept the experimental evidence alone as proof of this point. If a disorder of cholesterol metabolism is to have any effect on the

arteries, it must find some expression in changes in the blood, but evidence of the existence of such changes in association with the development of arteriosclerosis in man is entirely lacking. Indeed, belief in the participation of a disturbance of lipid metabolism in the etiology of human arteriosclerosis has always rested on little more than opinion and the nature of the supposed metabolic disturbance has never been clearly defined. No one considers it necessary to assume a disorder of calcium metabolism to account for the deposition of calcium in the walls of arteries¹⁷ even when this occurs in areas where there is no very obvious evidence of previous injury (e.g., Monckeberg's sclerosis), but there seems to be a general impression that a disturbance of lipid metabolism must be postulated to explain the accumulation of lipoids in the lesions of arteriosclerosis. This impression may prove to be correct, but there is no concrete evidence at present to show that it is. In any event, the cholesterol feeding experiments provide no valid reason for believing that a disturbance of cholesterol or lipid metabolism plays any part in the etiology of human arteriosclerosis. Moreover, there is no definite or concrete evidence from observations on human beings to support the idea. If a disturbance of lipid metabolism participates in the etiology of arteriosclerosis in man, the fact remains to be demonstrated in the future.¹⁸

The conclusions which have been drawn in the preceding paragraphs should by no means be interpreted as a deterrent to the further search for alterations in the blood to which importance could be attached in connection with the etiology of human arteriosclerosis. In the past, attention has been concentrated on possible quantitative changes in the lipid constituents of the blood plasma, but it may be that qualitative changes are more important. It is possible that alterations may occur in the physicochemical state of the lipoids, perhaps in their dispersion or in their relation to other colloids in the plasma. An interesting lead in this direction is furnished by the work of Alvarez and Neuschlosz¹ which suggests the possibility that the degree of saturation of the plasma with cholesterol may be of importance. Judging from their figures, one would conclude that the degree of saturation or of supersaturation is independent of the absolute quantity of cholesterol in the blood, and if this is the case, it would seem that the whole colloidal system of the blood plasma, complex as it is, would have to be brought into consideration.

(¹⁸) In his recent publications, Rosenthal (*Arch. Path.* **18**: 473 and 660, 1934) has discussed at great length the relationship of cholesterol metabolism to the development of arteriosclerosis. Like many previous authors, he has taken it practically as a foregone conclusion that disturbances of cholesterol metabolism constitute a factor of first importance in the etiology of the disease. However, he has not advanced any conclusive evidence whatever to support this contention, as careful examination of his data and of the references to the literature will show.

This is merely a suggestion. Other possibilities may be imagined, but the final solution of this problem must await the results of further investigation.

Before the subject of hypercholesteremia is dismissed, brief comment may be made on the results of the experiments in which interference with the reticulo-endothelial system or with the endocrine organs has been combined with cholesterol feeding. These results have been interpreted in certain quarters as indicating that the reticulo-endothelial system and the endocrine system are intimately concerned in the etiology of human arteriosclerosis. These conclusions do not necessarily follow. Again one is confronted with the necessity of making careful comparisons between the conditions associated with the development of experimental cholesterol arteriosclerosis and those with the development of human arteriosclerosis before conclusions are drawn. In the cholesterol feeding experiments in rabbits, the reticulo-endothelial cells become loaded with lipoids before any lesions make their appearance in the arteries, while in man no similar phenomenon is observed in connection with the development of arteriosclerosis. Now if the functional capacity of the reticulo-endothelial system in the rabbit is deliberately reduced, the development of experimental cholesterol arteriosclerosis may well be accelerated, but this constitutes no good reason for assuming that the same is true of human arteriosclerosis in which the reticulo-endothelial cells are not observed to participate in any way, and in which the etiologic conditions, especially as regards the rôle of the lipoids, are not capable of such close comparison. The assumption is rendered even more precarious by the lack of any confirmatory evidence from the study of human material.

Among the effects on the development of experimental cholesterol arteriosclerosis which have been attributed to the influence of various endocrine organs, those which follow interference with thyroid function are the best established and apparently the most definite. Hyperthyroidism and hypothyroidism, experimentally induced, tend respectively to retard and to accelerate the development of experimental cholesterol arteriosclerosis. As has been pointed out, the protective effect of the experimental administration of thyroid gland preparations is apparently related to an increased ability thus conferred on the rabbit to dispose of the excessive amounts of exogenous cholesterol introduced into the diet. The resulting hypercholesteremia is not nearly so extreme as in control animals fed on the same diet. To infer from this information that thyroid function plays a part in the etiology of human arteriosclerosis is unjustified, since the etiologic significance of hypercholesteremia in human arteriosclerosis is extremely doubtful, to say the least, and certainly is not comparable with that of hypercholesteremia in the experimental disease. Moreover even though hyper-

thyroidism and hypothyroidism in man have been recognized for a long time, and although it is now known that each has a characteristic effect on the level of cholesterol in the blood,¹¹ it has never been shown or even suggested that the development of arteriosclerosis in man is affected either favorably or unfavorably by these disturbances of thyroid function. In view of these facts, the experimental data cannot be regarded as valid evidence of the participation of the thyroid gland in the etiology of human arteriosclerosis. The experimental results obtained through interference with other organs of internal secretion are of equally doubtful significance.

The effect of iodides is similar to that of preparations derived from the thyroid gland. The administration of iodides reduces the intensity of the hypercholesterolemia which follows cholesterol feeding in rabbits and it retards the development of lesions in the arteries. It seems clear that the iodides exert their influence through an effect on thyroid activity, for their administration is without result in thyroidectomized animals. Thus, once again, the production of the experimental result is chiefly dependent on the effect of thyroid activity in facilitating the disposal of exogenous cholesterol. Therefore the arguments advanced in the preceding paragraph apply here, too, with equal force and render it impossible to predict with confidence on the basis of the experimental findings that the administration of iodides would be of any value in the prevention or treatment of arteriosclerosis in man. Put to the test in the treatment of human arteriosclerosis through the course of many years, iodides have not proved of definite therapeutic value. "It is very probable, as pointed out by Cushny, that the good effects of iodine when observed at all were noticed in cases in which arteriosclerosis was associated with syphilis" (Wyckoff¹⁸⁶).

(c) *Injury to the Arteries*—In section VI *f* and in section VIII of this paper, reasons have been given for the conclusion that the occurrence of injury to the walls of the arteries is an essential factor in the development of experimental cholesterol arteriosclerosis in rabbits. It has also been shown that the occurrence of local injury is the primary event, which is followed subsequently by the precipitation of lipoids in the injured area and by cellular proliferation in the intima. Almost all of the considerations on which these conclusions were based apply quite as appropriately in human arteriosclerosis. There is every reason to believe that local injury to the walls of the arteries is an essential factor and the primary event in the development of arteriosclerosis in man. Not only is this suggested by the experimental results, but all pertinent facts concerning human arteriosclerosis are consistent with this conclusion. It will hardly be necessary to repeat in full detail the arguments which have been set forth in discussion of this point in connection with the etiology of experimental cholesterol arteriosclerosis.

However, I may recapitulate briefly, applying the same lines of reasoning to the development of arteriosclerosis in man, and commencing as before with a consideration of the process by which lipoids accumulate in the walls of the arteries

The lipoids which appear in the walls of the arteries in human arteriosclerosis accumulate in such large quantities that they can hardly be considered as the products of the breakdown of tissue at the sites of the lesions. Of course, a small part of the lipid material may arise in this way, but it is clear from chemical analyses of human arteries that the bulk of the lipoids must come from elsewhere⁴ and therefore must be brought by way of the blood stream. It is evident that the lipoids contained in the blood plasma enter the arterial wall with the nutritive tissue fluid which is derived from the plasma. This fluid must be the immediate source of the lipoids which accumulate in the lesions of arteriosclerosis.

Since the function of the nutritive fluid is to nourish the tissue of the walls of the vessels, it is obvious that it must permeate freely through all parts of the walls. It seems highly probable that this fluid normally carries with it the various lipoids which are contained in the blood plasma. Nevertheless, lipid deposits do not occur in the walls of normal arteries, and as has been pointed out, they do not occur in normal human arteries even when there is prolonged hypercholesteremia which presumably increases the lipid content of the nutritive fluid. In arteriosclerotic vessels, lipid accumulations are not formed everywhere diffusely but only in localized and restricted areas. The latter fact could not be explained by an elevation of the lipid content of the blood plasma and of the nutritive fluid even if hyperlipemia could be shown to occur in regular association with arteriosclerosis in man. Whether or not there occurs any change in the lipoids of the blood in human arteriosclerosis, it is evident that the local conditions which exist in the wall of an artery at the sites where lipoids are deposited are different from those which obtain in the normal parts of the same artery where no lipid deposits occur. The local conditions which exist in the arterial walls at the sites of lipid deposition and which do not exist elsewhere must constitute the immediate cause of the actual precipitation of lipoids from the colloidal state in which they enter the walls of the vessels with the nutritive fluid. It is equally obvious that the appropriate local conditions must exist before any precipitation of lipoids takes place.

The possibility that the appropriate local conditions necessary for the precipitation of lipoids exist normally in certain parts of normal arteries has been mentioned in the discussion of the pathogenesis of experimental

(4) 136, 175, 182

cholesterol arteriosclerosis in rabbits. This suggestion was dismissed since it was inconsistent with the known facts and was supported only by the results of experiments which evidently had been misinterpreted. The idea is equally inconsistent with the facts concerning human arteriosclerosis so that it can be dismissed here, too, without further discussion. The only alternative and really the only reasonable conclusion is that the local conditions in the walls of the arteries which are responsible for the precipitation of lipoids develop as the result of changes in the walls of the arteries themselves. These local alterations which must occur before the deposition of lipoids becomes possible constitute the initial stage of the development of arteriosclerosis in man.

Just as in the case of the rabbit, the following general statement can be made. The occurrence of localized lipid deposits in the walls of the arteries is dependent on the coexistence of two sets of conditions. One set includes the conditions which must obtain in the blood and secondarily in the nutritive fluid which permeates the walls of the vessels. These are the general conditions. The second set comprises those conditions which must exist locally in the walls of the arteries at the sites where lipid deposits are to occur. These are the local conditions. When both the local and the general conditions are suitable lipid deposits develop.

In the case of the human being in contrast with the rabbit it seems probable that the general conditions are satisfied in the normal person. There is no evidence to show, nor any reason to believe, that an elevation of the blood lipoids is necessary to the development of human arteriosclerosis, and no other changes in the blood have ever been demonstrated in constant association with arteriosclerosis in man. One is justified, therefore, in drawing the tentative conclusion that the general conditions necessary for the formation of lipid deposits in the walls of the arteries are normally existent in the normal human being. On the other hand, corresponding with the state of affairs in the rabbit, the necessary local conditions almost certainly do not exist normally in man. As I have pointed out, the occurrence of preliminary local alterations in the walls of human arteries prior to the formation of lipid deposits can hardly be doubted, and indeed may be regarded as an established fact. This is admitted by almost every one, although it is seldom emphasized. Thus, with respect to the primary local changes in the arterial walls, experimental cholesterol arteriosclerosis in the rabbit is quite capable of comparison with arteriosclerosis in man. The important difference lies in the fact that in the human being one is forced at present to regard the occurrence of the appropriate local alterations in the walls of the arteries not only as the initial stage of the development of arteriosclerosis but as the one abnormal factor responsible for the subsequent train of events.

To determine the cause and nature of these initial local changes in the arterial walls is obviously a problem of paramount importance in the investigation of human arteriosclerosis. So far as these changes are concerned, comparable conditions seem to obtain both in the development of experimental cholesterol arteriosclerosis and in that of human arteriosclerosis, so that the experimental data may properly be drawn on for assistance in the elucidation of this problem. The experimental studies on cholesterol-fed rabbits have been misleading in some respects, for reasons which I have attempted to make clear. This fact, however, constitutes no argument for rejecting the conclusions which can be applied with justification. Therefore, one can accept as valid the experimental evidence which bears on the important question under consideration. As I have shown, the experiments yield evidence which suggests very strongly that some sort of injury is responsible for the preliminary local changes in the walls of human arteries which are essential to the subsequent formation of lipoid deposits. Many of the experimental measures which have been employed for the production of injury to the arteries of rabbits seem to have no direct significance as applied to man, but this does not diminish the weight of the evidence in support of the general conclusion that the primary local alterations in the arterial walls which lead to the subsequent development of the lesions of arteriosclerosis are the result of some sort of injury to the arteries, an injury to which in man the intimal layer seems to be especially susceptible.

That this inference is consistent with the knowledge of arteriosclerosis as it occurs in man is attested by the fact that many investigators have reached essentially the same conclusion from the study of human material alone. The interpretation of the experimental data which is offered here is decidedly new, but the conclusion as applied to human arteriosclerosis does not by any means constitute an original suggestion. Thus, the real contribution of the experiments is that they provide new and strong support for an old and well founded idea.

In the human being there are fewer opportunities than could be desired to observe the effects of various arterial injuries of known origin and of a kind which might be expected to produce lesions capable of comparison with those of ordinary arteriosclerosis. The best example at one's disposal is the injury produced by syphilis of the aorta. It is well known, of course, that syphilis affects primarily the adventitia and media of the aorta, but in addition, the intima is secondarily injured. As a result of this injury, intimal lesions develop which may simulate very closely those of arteriosclerosis. This is most often the case when the syphilitic aortitis is not too recent in origin, that is, when the injury has persisted for a sufficient length of time to permit the relatively slow process of lipoid deposition in

the intima to become apparent. Such lesions, like those of ordinary arteriosclerosis, are characterized by a fibrous thickening of the intima and by more or less abundant accumulations of lipoids which are found partly in association with the intercellular material in the thickened intima and partly within foam cells.

Occasionally cases are seen at autopsy in which such "arteriosclerotic" lesions are found in the intima overlying the portions of the aortic wall which are affected by syphilis, while the remaining parts of the aorta and the other arteries throughout the body are practically free from arteriosclerosis. Evidently the injury to the wall of the vessel in these cases is responsible for the development of the arteriosclerotic changes associated with the syphilitic aortitis, for the uninjured parts of the aorta and the arteries elsewhere remain unaffected, although they are exposed to identical conditions so far as possible changes in the blood are concerned. Apparently, then, in the human being a suitable local injury to the intima is capable of producing intimal lesions which possess the essential characters of ordinary arteriosclerosis. The occurrence of injury which is the primary event, is followed subsequently by the precipitation of lipoids and by cellular proliferation in the injured intima. Probably the same is true of arteriosclerosis as it occurs quite apart from injuries of such well recognized origin.

The cause of the arterial injury which initiates the development of human arteriosclerosis is obscure and the cholesterol feeding experiments provide hardly any suggestive information bearing on this problem. In the past it has generally been taken for granted, on the basis of the experimental results, that hypercholesteremia in man has the ability to injure the arteries, but it has been shown here that the experimental data fail to demonstrate that this is true even in rabbits. The question of the real cause of the primary injury to the arteries in experimental cholesterol arteriosclerosis has never been carefully investigated. The evidence obtained in human beings, far from incriminating hypercholesteremia as a cause of arterial injury, indicates rather that hypercholesteremia of itself has no damaging effect on the arteries.

Most of the forms of experimental injury which have been employed as accessories to cholesterol feeding in rabbits seem to have no direct significance as applied to arteriosclerosis in man. The only exception of possible importance is the arterial injury produced in rabbits by intravenous injections of certain bacteria (see section VI *f* of this paper). It should be borne in mind, however, that the influence of bacterial infections on the development of experimental cholesterol arteriosclerosis is really not well established. The available experimental evidence bearing on this point is so meager that little importance can be attached to it at present. Moreover, caution must be exercised in the interpretation of such experiments for it is well known that the

arteries of rabbits are extraordinarily susceptible to injury. Toxic agents which are injurious to the rabbit's arteries may not be equally injurious in man. Studies of human material should be more reliable, but they have led only to the formulation of a variety of opinions as to the rôle of infections in the etiology of human arteriosclerosis. It has been thought for a long time that bacteria, perhaps through the agency of their toxic products, may cause injuries to the arterial walls and thus may initiate the development of arteriosclerosis. This idea, although it has been favored by many students of arteriosclerosis, is difficult to prove, but is equally difficult to disprove. One can only say that the evidence which has been brought forward up to the present time is inconclusive.⁹⁷

The tendency of the lesions of human arteriosclerosis to develop around the mouths of branching arteries is said to indicate that the injury at these points is due to the stretching and distorting action of mechanical forces. The lesions of experimental cholesterol arteriosclerosis likewise tend to favor the areas near the orifices of branching vessels and the same conclusion might be drawn from this fact, but the experimental observations really add nothing new to the arguments which have been built on observations on human arteries. In both instances, lesions are distributed in many places at a distance from possible weak spots in the walls of the arteries, so that it is difficult to believe that mechanical forces are wholly responsible. It seems more likely that the areas about the mouths of branching arteries are merely somewhat more susceptible than other parts of the wall of the vessel to the action of the injurious agent which produces lesions elsewhere. Whether this special susceptibility to injury is dependent on the operation of mechanical forces or on the peculiar structure of the wall of the vessel at such points or on some other local peculiarity remains undetermined. The experiments of Harrison,⁵⁹ of which some account has already been given, do suggest that mechanical forces which produce excessive internal movements within the arterial walls around the orifices of branching vessels are responsible for the tendency of lesions to develop in these areas.

Many other suggestions have been made as to the possible origin of the injury to the arteries which is responsible for the development of human arteriosclerosis. A discussion of these possibilities would be fruitless, however, for it is impossible at present to demonstrate that any one of the suggested causes of arterial injury is of decisive significance in the etiology of arteriosclerosis in man. One would suppose that the appropriate local injury must be one of moderate intensity which probably persists over a considerable period of time or which perhaps is repeated at intervals, but what it is that produces such an injury is really quite unknown.

The necessity of preliminary local alterations in the walls of vessels as a factor indispensable to the precipitation of lipoids and to the subsequent development of the lesions of human arteriosclerosis is perfectly clear, and there is every reason to believe that injury to the walls of the arteries can produce these changes. However, Aschoff¹⁶ especially has emphasized the importance of primary alterations in the arterial walls which seem to occur simply as a part of the process of aging and which he believes are manifested chiefly in the intercellular cement substance or ground substance. Wells¹⁷ has placed great emphasis on the deterioration of elastic fibers, a change which he has attributed to "aging" of the colloidal elastin of which these fibers are composed. Such alterations in the arterial walls undoubtedly occur in old age, but it is difficult to believe that the process of aging plays a part of any consequence in the development of arteriosclerosis in relatively young people. The importance which one can attach to the changes attributable merely to chronological old age limits itself automatically to the later decades of life. One can say only that the appropriate local alterations in the arterial walls, if they are not produced prematurely by local injury, will come eventually as a manifestation of age. So far as aging is a biologic property inherent in the human organism, it must be inevitable and its consequences therefore, are overshadowed in importance by those for which some form of injury to the walls of the arteries can be held responsible. Accordingly the question of the cause or causes of this arterial injury still presents itself for further investigation as the most important problem in the etiology of human arteriosclerosis.

Attention may now be directed again to the lipoids which accumulate in the lesions of arteriosclerosis. The exact nature of the process by which lipoids come to be deposited secondarily in damaged areas in the walls of arteries has never been satisfactorily explained. The experimental observations indicate that changes in the intercellular ground substance are especially important in this connection, but no information is forthcoming as to the intimate nature of these alterations beyond the fact that they can be produced by injury. Neither is there any information available as to the manner in which the local changes can effect the precipitation of lipoids from the colloidal state in which they enter the walls of the arteries with the nutritive fluid. It is said that the lipoids are adsorbed by the altered intercellular material but all of this is really obscure, a fact which emphasizes the necessity for further studies directed toward the clarification of this complex process.

Following the original injury to the wall of the vessel, when lipid deposits begin to make their appearance, it becomes a question whether or not the lipoids in the intima can themselves act as injurious agents and thus propagate the arteriosclerotic process. It is probable that the original injury continues to act for a time at least, and the same injury

may be fully responsible for the whole development of the lesions, while the precipitation of lipoids in the injured intima may be entirely incidental. However, it seems highly probable that the precipitated lipoids, and especially the cholesterol esters which are apparently the most difficult of removal, play a part, and perhaps an important part, in the subsequent development of the arterial lesions. It is clear from the study of both the experimental and the human material that the lipoids deposited in the injured intima stir into activity the numerous macrophages which are attracted to the site and which then engulf a large part of the lipid material. It seems entirely probable, too, that the free lipid deposits can stimulate the proliferation of fibrous connective tissue cells in the intima. This effect is added to that of the original injury to produce a reparative fibrous tissue reaction in the affected areas. If the original injury which initiated the whole process ceases to operate, it is probable that the lipid deposits prevent immediate healing and continue to exert their influence so that the lesions persist, progressing slowly. If this is true, the presence of lipid deposits really renders the lesions of arteriosclerosis self-propagating. However, the experimental evidence indicates that the lipoids may be slowly removed under certain conditions, among which the withdrawal of the source of arterial injury is probably the most important. One can infer, therefore, that in human arteriosclerosis the termination of the influence of the original arterial injury may permit a gradual absorption of the lipid deposits which, if they are not already too extensive, may even disappear finally, leaving only a fibrous thickening of the intima.

XI SUMMARY AND CONCLUSIONS

Arterial lesions which resemble those of human arteriosclerosis can be produced in rabbits by the administration of diets containing considerable quantities of cholesterol. I have chosen to call this experimental disease of the arteries "experimental cholesterol arteriosclerosis." The literature bearing on experimental cholesterol arteriosclerosis is reviewed in detail. The results of attempts to produce arterial lesions by cholesterol feeding in animals other than rabbits are described. The data which have arisen from all of these experiments are summarized briefly in section VII of this paper.

On the basis of these data, the etiology and pathogenesis of experimental cholesterol arteriosclerosis in the rabbit are discussed. It is concluded that the presence of considerable quantities of cholesterol in the diet with a resulting elevation of the level of cholesterol and other lipoids in the blood is essential to the development of the typical arterial lesions in rabbits. It is shown, however, that hypercholesterolemia alone cannot be regarded as the cause of the lesions in the arteries. There are preliminary local alterations in the walls of the arteries which pre-

cede the precipitation of lipoids. Evidence is advanced to show that these preliminary changes are due to some form of injury to the arteries attendant on the experimental procedure of cholesterol feeding. It is concluded that the occurrence of local changes in the arterial walls, due in all probability to injury of some kind, is the primary event in the development of the lesions of experimental cholesterol arteriosclerosis, an event which is followed subsequently by the precipitation of lipoids in the injured areas.

As a preliminary to the discussion of the significance of the experimental results, a comparison is drawn between the anatomic lesions of experimental cholesterol arteriosclerosis and those of human arteriosclerosis. It is demonstrated that the two diseases are not identical and that there are a number of important differences between them. These differences are of such a nature as to suggest strongly that the role of the lipoids in the development of arteriosclerosis is greatly exaggerated in experimental cholesterol arteriosclerosis as compared with arteriosclerosis in man. A number of normal differences between the rabbit and man which render interpretation difficult and uncertain are pointed out. It is shown that the experimental data are not capable of accurate interpretation without recourse to data on corresponding points derived from the study of human material. With this fact in mind, an attempt is made to correlate the data concerning experimental cholesterol arteriosclerosis with the available information regarding human arteriosclerosis. The three main factors which can be recognized in the etiology of experimental cholesterol arteriosclerosis, namely, cholesterol in the diet, hypercholesteremia and injury to the arteries, are considered in order as regards their possible significance in the etiology of human arteriosclerosis. With full cognizance of the inferences which have been drawn in the past from the experimental results the following conclusions are reached for reasons which are given in their appropriate places.

The results of cholesterol feeding experiments in rabbits do not constitute a valid reason for believing that an excess of cholesterol in the diet plays any rôle in the etiology of arteriosclerosis in man. No convincing evidence in support of such a belief exists at present.

Hypercholesteremia is not found with any regularity in association with human arteriosclerosis. It seems highly probable that arteriosclerosis in man can and usually does develop without deviation of the cholesterol content of the blood beyond the normal limits of variation. In any event, there is no valid evidence to support any other conclusion.

Hypercholesteremia of itself cannot be regarded as a cause of human arteriosclerosis. Although hypercholesteremia, when it occurs, might be expected on theoretical grounds to accelerate the development of

arteriosclerotic changes which have already been initiated, there is little evidence at present to indicate the existence of such an effect

The cholesterol feeding experiments provide no valid reason for believing that a disturbance of cholesterol or lipid metabolism plays any part in the etiology of human arteriosclerosis. There is no definite or concrete evidence from observations on human beings to support the idea. If a disturbance of lipid metabolism participates in the etiology of arteriosclerosis in man, the fact remains to be demonstrated in the future.

The initial stage in the development of human arteriosclerosis consists of local changes in the walls of the arteries themselves, changes which are responsible for the subsequent precipitation of lipoids in the affected areas. In man, as in the rabbit, there is every reason to believe that these changes follow as the result of some sort of injury to the arterial walls. As to the cause of this injury, the experimental data yield hardly any information which is capable of application in the human being. Some possible causes of the arterial injury which have been suggested previously and on which the experimental results have some bearing are discussed briefly. Brief comment is also made on the influence of age. No definite conclusions are reached, since it is clear that the cause of the injury to the arteries which is responsible for the development of human arteriosclerosis is unknown.

Following the initial local injury, lipoids are deposited in the injured intima, especially in the intercellular substances. These deposits stir into activity numerous macrophages which are attracted to the site and which then engulf a large part of the lipid material. It seems entirely probable that the free lipid deposits can stimulate the proliferation of connective tissue cells in the intima. This effect is added to that of the original injury to produce a reparative fibrous tissue reaction in the affected areas. If the original injury which initiated the whole process ceases to operate, it is probable that the lipid deposits prevent immediate healing and continue to exert their influence so that the lesions persist, progressing slowly. Under these conditions, however, it seems likely from the experimental evidence that the lipoids may be slowly removed and may even disappear finally, leaving only a fibrous thickening of the intima. It is unnecessary to assume that a disturbance of cholesterol or lipid metabolism plays a part in any stage of the process.

This outline of the development of human arteriosclerosis is entirely consistent with the knowledge derived from the study of arteriosclerosis in man, and at the same time it incorporates those inferences which can be drawn with justification from the experimental data. It seems to be the most reasonable working hypothesis which can be constructed from the evidence available.

A tentative scheme of the relationship between experimental cholesterol arteriosclerosis and human arteriosclerosis is offered which explains in a logical way the apparent discrepancies between the conclusions which have been drawn in this paper regarding the two diseases

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Notes and News

Society News—The Third International Congress of Comparative Pathology will be held in Athens, Greece, on April 15 to 18, 1936. Special reports will be made on nephrosis, amyloidosis, leishmaniasis, spirochetosis and avitaminosis. Information may be obtained from Prof. Anthony Codounis, 40 Didotou Street, Athens.

Grants in Aid of Research—Applications for grants in aid of research for 1936 by the American Association for the Advancement of Science must be received at the permanent secretary's office in Washington, D. C., on or before October 30 next.

The Division of Medical Sciences of the National Research Council will hold a special meeting in November 1935 for the consideration of applications for grants-in-aid in this field. Applications to be considered at this meeting must be on file with the secretary of the committee on grants-in-aid, Dr. Clarence J. West, not later than Oct. 1, 1935. Applications received after Oct. 1 and prior to Feb. 15, 1936, will be acted on at the next regular meeting of the committee in March 1936.

Resolution in Regard to the Coroner's Office—At the annual meeting of the Illinois State Medical Society, on May 21 to 23, 1935, the following resolution was adopted by the house of delegates:

WHEREAS, the Coroner's duties are both magisterial and medical, each of these requiring a high degree of specialized knowledge, and

WHEREAS, the Coroner has no qualifications except political ability to get votes, not being required to be either lawyer or doctor, and

WHEREAS, the Coroner does nothing that must not be done over again, and

WHEREAS, There are grand and petit juries to handle the work of the Coroner's jury, and

WHEREAS, The office of Coroner is both useless and costly, and

WHEREAS, The press is heartily in sympathy with this move as evidenced by editorials such as "The Office of Coroner" (*Chicago Daily News*, April 7, 1933), "What Price Gangster Protection" (*Chicago Daily News*, Oct. 7, 1933), "An Outrageous Verdict" (*Chicago Daily Tribune*, April 1933), and many other editorials and news stories appearing from time to time,

BE IT THEREFORE RESOLVED, That the Illinois State Medical Society go on record as favoring the abolition of the Coroner's office and establishing in its stead a Medical Examiner who is a licensed doctor of medicine, to be appointed by the governor or civil service commission, whose duties will be medical examination only to determine causes of death, the legal investigative procedures to be left to the district or state attorneys,

AND BE IT FURTHER RESOLVED, That copies of this resolution be sent to the Illinois State Bar Association and to the Press.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Jan 23, 1935

KORNELI TIRPIAN, *President, in the Chair*

W. F. JACOBS, *Secretary*

THE FIBER AND NUCLEAR STRUCTURES IN A CASE OF HYPOPLASIA OF THE CEREBELLUM GILBERT BICK

A 9 months old child died after the making of an encephalogram. The cerebrum was normal in size, cell and fiber structure. The cerebellum, on the other hand was found to be long and narrow, though it contained the normal number of lobules and sulci. It resembled the cerebellum in miniature described by Nonne. Serial sections were made of one half of the cerebellum in the transverse direction and of the other half in the sagittal plane and stained by the Weigert-Pal method.

Cerebellum Proper—The myelin of both the vermis and the flocculus was well stained. The lobules of the hemisphere, however, were myelinated in varying degrees. The tonsilla was most poorly myelinated, the biventer not so poorly, while the myelin of the lobuli gracilis and quadrangularis was somewhat better retained. The lobuli semilunares superiores and inferiores were as deeply stained as the vermis.

The pars intermedia was confirmed. It demonstrated a connection between the hemisphere and the emboliform nucleus.

Brain Stem Nuclei and Tracts Associated with the Cerebellum—The nuclei arcuati and the stria Piccolomini were absent. The inferior olive and the accessory olives were normal. Fibers from these structures could be traced swinging above the dentate nucleus into the lobuli semilunares. This is contrary to Holmes' and Stewart's schematic representations as well as Brouwer's conclusions.

All pontile nuclei other than the peripeduncular, intrapeduncular and reticular groups were absent. The stratum superficialis pontis as well as the fibrae rectae pontis were well retained. This seems to prove that this group of fibers and the reticular nucleus are more ancient than the rest of the arms of the pons.

Cerebellar Nuclei Proper—(a) The dentate nucleus showed the macrogyric ventral sheath more embryonic than the microgyric dorsal sheath. The latter also was immature, giving few if any fibers to the superior peduncle.

(b) The emboliform, or frontal conglomerate, nucleus was composed of very large cells and extremely dense fiber structures. This nucleus was the sole source of origin of the dentatorubrothalamic pathway.

(c) The nuclei globosi and fastigii appeared normal.

Cerebellar Connections—The middle peduncle with the exception of the stratum superficiale was entirely aplastic.

The superior peduncle originated from the emboliform nucleus and decussated in the brain stem to form the dorsal portion of the commissure of Werneckinck, thus confirming Hatchek's experimental work. The restiform body and the ventral spinocerebellar tract appeared normal.

A definite and unmistakable connection between the mesencephalic trigeminal root and the cerebellum was confirmed, in sagittal sections many fibers could be traced emerging from the trigeminal root into the cerebellum.

It seems that the hypoplasia of the cerebellum may have been due to a lack of proper neurobiotaxic stimulation because the arm of the pons was not normally developed. Two conditions are offered in confirmation of this view.

(a) Fetal cerebral atrophy causes contralateral cerebellar atrophic changes, whereas atrophy of the adult cerebrum (Pick's disease, porencephaly, etc.) in most cases does not cause these changes. Were it just a matter of the amount of fibers, a difference between "secondary" changes following fetal disturbances, on the one hand, and atrophy of the adult cerebrum, on the other, would not be present. In fetal life the contralateral atrophy must be regarded in the light of a lack of the proper neurobiotaxic stimulation for "massive" development.

(b) In the semilunar lobes, in which the myelination was very splendidly retained through the olivocerebellar fibers, the lobules were, however, still very small, no larger than the adjacent lobules, in which the amount of myelin was scant. The well myelinated semilunar lobules also lacked the neurobiotaxic stimulation necessary for their development in the large, "fleshy" lobules of the hemisphere that one knows of normally.

Regular Meeting, Feb 16, 1935

KORNEL TERPLAN, *President, in the Chair*

W F JACOBS, *Secretary*

DEMONSTRATION OF SUCROSE IN THE CEREBROSPINAL FLUID OF A PATIENT WITH ACUTE ENCEPHALITIS ROGER S HUBBARD and KORNEL TERPLAN

The effect of sucrose injected intravenously on the pressure of the cerebrospinal fluid has recently been described (Bullock, L T, Kinney, R, and Gregerson, M I *Am J Physiol* **109** 17, 1935). When concentrated solutions of the sugar are injected intravenously a fall in intracranial pressure occurs. Later there is a gradual return to normal values without the secondary rise which is sometimes noted when dextrose is used.

In the course of the treatment of a patient with encephalitis 250 cc of a 26 per cent sterile solution of the sugar was injected to decrease the intracranial pressure. The patient was a 7 year old boy who had enjoyed perfect health until about three weeks prior to his death, when a cold in the head with severe cough developed. Although there was no whooping or vomiting, two doses of therapeutic pertussis vaccine were given. Only in the last three days of life the temperature rose to 101 F. The boy felt chilly and complained of headache and dizziness. General convulsions developed, followed by deep coma. There was slight rigidity of the neck with a positive Babinski sign on the right side. The spinal fluid showed 56 cells but was clear. The child died showing symptoms of pulmonary edema six hours after the intravenous injection of sucrose. The clinical diagnosis was "acute encephalitis (?)"

The autopsy was performed six hours after death. The brain showed unusually marked swelling and hyperemia, its weight was 1,400 Gm. The amount of spinal fluid was distinctly decreased. Only 7 cc was collected from the basal cisternae. The ventricles contained very little fluid, they appeared markedly compressed by the swollen brain substance. Except for acute catarrhal tracheobronchitis, inflammatory edema of the lungs and recent lobular pneumonia there were no other noteworthy findings.

Histologically the brain showed an almost diffuse inflammatory process involving the white and the gray matter alike. There were moderately dense perivascular lymphocytic infiltrates, especially around small veins. In a few areas, e.g., the caudatum and the frontal lobe, the inflammatory process showed focal accentuation with small nodular infiltrates. The leptomeninges, too, showed slight focal infiltration with lymphocytes. The pons, the medulla oblongata and the upper part of the cervical cord were practically free from such infiltrations.

It seemed worth while to determine whether sucrose could be demonstrated in the fluid obtained at the postmortem examination. For this purpose a specimen of the fluid was treated with hydrochloric acid to give a concentration thirty-three hundredths normal and was then heated on a water bath for one-half hour to hydrolyze any disaccharide which might be present. The acid was then neutralized and the levulose determined by the method of J. H. Roe (*J Biol Chem* **107** 15, 1934). A control specimen obtained before the sucrose was injected was also treated in the same way, and the levulose content of both specimens was determined without submitting them to acid hydrolysis. The results of these analyses are given in the table.

Apparent Levulose Content of Spinal Fluid From a Patient with Encephalitis

Specimen	Milligrams of Levulose per 100 Cc	
	Before Injection of Sucrose	After Injection of Sucrose*
Hydrolyzed	3.4	15
Unhydrolyzed	3.4	10

* Specimen obtained after death

The figures show clearly that the fluid taken at the autopsy contained a substance which yielded levulose on acid hydrolysis. This substance was absent from spinal fluid taken before sucrose was administered. It seems practically certain, therefore, that sucrose was present in the fluid. The concentration of sucrose can be calculated approximately from the formula

$$(15-3.4) \times \frac{\text{molecular weight sucrose}}{\text{molecular weight levulose}} = 22 \text{ mg sucrose per 100 cc of fluid}$$

Because Roe's method gives positive results with unhydrolyzed sucrose as well as with levulose, and because spinal fluid contains an unexpectedly high concentration of levulose (Hubbard, R. S., and Garbutt, H. R. *Proc Soc Exper Biol & Med* **32** 986, 1935), further experiments were carried out in an attempt to devise a more satisfactory method for the determination of sucrose in the spinal fluid. It was found possible to do this by incubating spinal fluid with one of the strains of *Bacterium coli* which ferments fructose but does not attack sucrose, subjecting the incubated material to acid hydrolysis and carrying through Roe's procedure. When this technic was applied in further experiments the entrance of sucrose into the spinal fluid after the injection of the sugar into the blood stream was confirmed.

Regular Meeting, March 27, 1935

KORNEL TERPILIAN, *President, in the Chair*

W. F. JACOBS, *Secretary*

SOME IMMUNOLOGIC AND SEROLOGIC PROBLEMS IN TUBERCULOSIS ERNST WIT-
EBSKY, Heidelberg and New York (by invitation)

While diseases such as scarlet fever are followed by definite immunity against reinfection, it is doubtful whether immunity against chronic infectious diseases such as tuberculosis and syphilis exists. Since the time of Robert Koch it has been known that a tuberculous animal will react to a second infection (reinfection) differently from a normal one. A lesion will appear as early as a few hours after reinfection. It vanishes a few days later and is not followed by a general infection. This typical behavior, also called "infectious immunity," is due to the presence of an active tuberculous lesion in the body. "Infectious immunity" means immunity during the stage of actual infection. The "infectious immunity"

in tuberculosis can be compared to that in syphilis, in which reinfection does not induce a primary lesion at all. The failure of the syphilitic person to acquire a primary lesion is due to the presence of living spirochetes somewhere in the body. After complete cure by antisyphilitic treatment, however, it is possible again to produce a primary lesion as in noninfected persons.

Regarding the striking similarity between the Koch effect on the one hand and the Arthus phenomenon on the other, many authors maintain that the tuberculin test is based on an antigen-antibody reaction. However, there are important differences between both. It is possible to transfer the Arthus phenomenon by means of the serum of a sensitized person to a normal one. The attempt at passive transfer of the hypersensitiveness against tuberculin fails. Neither does tuberculin (the bacteria-free filtrate of cultures of tubercle bacilli) induce the formation of antibodies, nor is it possible to prove the existence of antibodies against tuberculin in the serum by precipitation reactions or complement fixation. Thus, tuberculin lacks all the qualities which characterize an antigen. In spite of recent important investigations carried out by Seibert and by Long concerning the chemical nature of tuberculin it must be stated that the basis of the tuberculin reaction is still a mystery of nature.

The experimental analysis of serum in tuberculosis shows two different changes: (1) an alteration of the albumin-globulin ratio, (2) the presence of specific antibodies. Many reactions are described which are based on the increased lability of plasma and serum in tuberculosis. Reagents such as alcohol, saline solutions, distilled water and some chemical reagents are used. The increase of lability sometimes parallels the intensity of the actual process. The change in the albumin-globulin ratio is, however, not specific for tuberculosis but occurs also in such conditions as carcinoma, acute infectious diseases and pregnancy. The presence of real antibodies in the serum of tuberculous people may be demonstrated by using specific antigens only, e. g., tubercle bacilli or certain substances derived from tubercle bacilli. By means of the specific absorption test it is possible to prove the presence of antibodies in the serum of patients suffering from tuberculosis. During the last few years several new antigens and reactions for the serodiagnosis of tuberculosis have been described, based mostly on complement-fixation methods. Their sensitivity as well as specificity has improved very much. The experience derived from these methods shows that, especially in adults suffering from phthisis of the lungs, a high percentage (about 80 per cent) of positive results is obtained. On the other hand, in the so-called second Ranke's stage of tuberculosis, e. g., tuberculosis of the skin, bones, joints and peritoneum, the percentage of positive reactions obtained is rather low (25 per cent or less). The complement-fixation test in tuberculosis may be of differential diagnostic value when antigens the specificity as well as the sensitivity of which is satisfactory are employed. The test is also suitable for the examination of pleural fluids and may be of great help in deciding the question whether or not a pleural fluid is tuberculous. It is difficult thus far to make a statement concerning the prognostic value of the presence of antibodies in the serum in tuberculosis. A rather high percentage of serums from dead bodies contain antibodies. Thus they do not necessarily inhibit the progress of the disease. Further investigations are necessary if one is to answer the question whether the presence of antibodies after successful pneumothorax treatment is due only to the persistence of antibody functions or to the fact that tubercle bacilli are still present somewhere in the body.

Tuberculin reactions, lability reactions and antigen-antibody reactions in tuberculosis are independent of each other. Although the definite interpretation of these biologic tests is still a subject for further study, their suitable application may already be of value for clinical purposes.

ISOLATION OF A TUBERCULIN-LIKE SUBSTANCE FROM THE URINE OF PATIENTS WITH ACTIVE TUBERCULOSIS AND ITS PRACTICAL APPLICATION AS AN AID IN DIAGNOSIS. E. B. HANAN

These studies are based on the principle that a tuberculin-like substance of specific antigenic nature is excreted in the urine during the active stage of tuber-

culosis. When concentrated and partially purified this material may be used in conjunction with old tuberculin as an aid to distinguish between the latent and the active phases of the disease. Vacuum-concentrated and dialyzed urinary extracts were obtained in 124 cases of tuberculosis. By the Mantoux technic, 0.1 cc of the extract was injected near the site of a similar injection of a 1:10,000 dilution of old tuberculin on the forearm of the patient from whom the urine was obtained. In 107 of these cases active tuberculosis was proved to be present. The reaction to old tuberculin was questionable in 7 and negative in 9. Seventeen patients were proved not to have tuberculosis. The old tuberculin provoked no reaction in 10, the auto-urinary extract, in 15. Twenty normal persons gave negative reactions to auto-urinary extract.

In further studies it was observed that a chloroform-ether mixture extracted from a water solution, slightly alkaline or acid in reaction, one tenth of a known amount of ultraprotein tuberculin. This method provided a more simple procedure than the vacuum concentration and dialyzation method. A modification using chloroform-ether extraction was applied as a routine in 166 cases of suspected tuberculosis. In 44 of the cases the disease was proved to be active tuberculosis. Old tuberculin gave a negative result in 1 and the auto-urinary chloroform-ether extract a negative result in 5. In 64 of the cases active tuberculosis could not be proved. The reaction of old tuberculin was negative in 51 and positive in 13.

These results were not entirely gratifying but indicate the importance of this kind of investigation. Efforts were made to isolate the active principle in a more highly concentrated and purified form in order to test its specific antigenic nature. In studies on tuberculin precipitation it was observed that the optimum hydrogen ion concentration at which ultraprotein tuberculin precipitated was pH 2.8. It was further observed that when urine from persons with active tuberculosis was adjusted to pH 2.8 turbidity frequently developed. This reaction was not specific, but when it occurred in a case of tuberculosis the turbidity was noted to increase in relation to the severity of the disease. Urine showing marked turbidity was collected from a tuberculous patient to the amount of 1,000 cc, and the tuberculin-like substance was concentrated and purified by a benzoic acid method. This material proved to be highly antigenic, giving specific tuberculin reactions and specific fixation of complement with serums from patients with active tuberculosis and from immunized rabbits.

Further studies are being carried out on the isolation of this tuberculin-like substance from urine by absorption methods.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

THE COURSE OF RHEUMATIC HEART DISEASE A C DeGRAFI and C LINGG,
Am Heart J 10 459, 1935

The course of rheumatic heart disease is described as observed in 644 patients who are dead in a total of 1,633 patients coming under observation in the ten years up to 1931. Of these patients 55.8 per cent were males and 44.2 per cent were females. Rheumatic heart disease usually existed alone (94.5 per cent) and was seldom combined with other etiologic types (5.5 per cent). This disease was in the main one of childhood and early adult life, for it occurred and ran its course chiefly within the first four decades of life. The proportion of sufferers more than 40 years of age was small. Three-fourths of those who survived to adult life were dead before the age of 40. The average age at the time of the initial infection was 17 years, that at the time of the first symptoms of cardiac insufficiency was 28 years, that at the first appearance of heart failure was 30 years, and that at death was 33 years. Stated in another way, the average patient was infected at the age of 17 but was free from symptoms and able to carry on ordinary physical activity for eleven years. He then began to suffer from diminished cardiac reserve culminating in heart failure two years later. From this time on until death three years later he was wholly an invalid or at least, in most cases, seriously incapacitated. The period of economic usefulness of the person afflicted with rheumatic heart disease was, on the average, not more than eleven years after the initial rheumatic infection, in most cases it was less than nine years. Once symptoms of cardiac insufficiency appeared heart failure and death followed rapidly. Fifty per cent suffered their first symptoms and failure and died within a period of from sixteen to twenty years, or between 20 and 40 years of age, the years of early maturity. To see even terminal stages of this disease after the age of 50 years was not a common experience. Death usually occurred as the result of heart failure, but life was shortened in a fair proportion of cases by such conditions as subacute bacterial endocarditis, pneumonia and other diseases.

AUTHORS' SUMMARY

LATENT CARDIAC COMPLICATIONS FOLLOWING SYDENHAM'S CHOREA H SCHWARZ
and S D LEADER, Am J Dis Child 49 952, 1935

Seventy-five cases of so-called pure chorea were observed for from one to twelve years for evidence of cardiac involvement. The term "pure" is used to describe chorea in which no other manifestations of rheumatism were noted clinically. Cardiac involvement was diagnosed by physical signs, roentgenograms and electrocardiograms. The roentgenographic evidence was remarkably constant and corresponded well with the physical findings, occasionally, positive roentgenographic evidence was found in cases in which the physical findings were of no significance. The longer the period of observation after the first attack of chorea the higher was the percentage of cardiac involvement. After seven or eight years the percentage apparently approached 100, although the number of cases studied is too small to permit a definite statement. The signs of cardiac involvement developed insidiously, without recognizable attacks of rheumatic fever and in some cases without further attacks of chorea. In this series aortic murmurs, pericarditis and subcutaneous nodules were never encountered. It seems probable that what is said of rheumatic fever may also be said of chorea. "The heart is always involved." This probable cardiac involvement is added evidence that Sydenham's chorea is of rheumatic origin.

FROM THE AUTHORS' SUMMARY

HEMORRHAGIC ENCEPHALITIS A B BAKER, Am J Path **11** 185, 1935

Twenty fatal cases of encephalitis with hemorrhagic changes in the brain are described. In one case the brain tissue was virulent on inoculation in the brains of rabbits. Baker believes that hemorrhagic encephalitis is a distinct disease.

ANTIGROWTH EFFECT OF LIPOID FRACTIONS OF TISSUE EXTRACTS F A MCJUNKIN and J W HENRY, Am J Path **11** 353, 1935

The lipoids of organs exert an antigrowth effect on the kidney and liver on injection into young rats. The action is potent, since the quantity contained in 0.5 Gm of fresh kidney is effective, the inhibiting substance is probably in the phospholipid fraction, it is not specific, is not limited in its action to one or more organs and is widely distributed in the body. An explanation of the function of lipoids in the regulation of cell growth must await investigation of other factors concerned in nuclear division.

THE CEREBROSPINAL FLUID IN EXPERIMENTAL POLIOMYELITIS OF MACACUS RHESUS MAURICE BRODIE and BERNARD WORTIS, Arch Neurol & Psychiat **32** 1159, 1934

Brodie and Wortis studied the spinal fluid of over one hundred rhesus monkeys experimentally inoculated with poliomyelitis virus and compared their observations with those on the spinal fluid of normal monkeys. In the early stages of the disease the spinal fluid usually showed predominance of polymorphonuclear cells, with the onset of paralysis there was an excess of lymphocytes. In some cases the cell count was normal, and it was not as a rule influenced by the stage of the disease (preparalytic, paralytic or extreme paralytic). In the mild non-paralytic type the cell count became normal in from three to ten days, and in severe forms, within the fourth week after the onset of the paralysis. Pleocytosis developed in from twelve to thirty-six hours after an incubation period of from three to seven days followed by a rise of temperature (2 to 6 F). Symptoms of involvement of the central nervous system appeared either immediately or within forty-eight hours or even later. An important pathologic observation was that though the meninges were involved the primary lesion was in the spinal cord. In general, the clinical types of experimental myelitis and their serologic features were similar to those observed in man. The respiratory quotient for animals intracerebrally inoculated with poliomyelitis was found to be normal—the spinal cord of such animals was able, for instance, to oxidize dextrose.

GEORGE B. HASSIN

CHANGES IN THE CEREBRAL CORTEX PRODUCED BY THERMOCOAGULATION J G DUSSER DE BARENNE and H M ZIMMERMANN, Arch Neurol & Psychiat **33** 123, 1935

Dusser de Barenne demonstrated that thermocoagulation of the cortex causes local destruction of the ganglion cells of the superficial lamellae. In acute states, with a survival period of from seven to twelve days, only the ganglion cells are destroyed, in chronic experiments, with the application of a temperature of 80 C and a survival period of four months, the glial and mesodermal elements also are destroyed. Aside from the time element, the degree of heat used is of importance. Thus, a temperature of 65 C applied locally to the cortex for two seconds causes destruction of two outer layers, applied for three seconds, it causes additional destruction of a portion of the third layer. A temperature of 70 C applied for three seconds destroys three outer layers and when applied for four seconds affects four outer lamellae. Application of a temperature of 50 C for half a minute also produces destruction of the ganglion cells, but there are marked reactive proliferation of the glial and fibroblastic elements of the upper three lamellae and preservation of some pyramidal cells of the deeper portion of the

third layer As chronic thermocoagulation of 80 C produces in contrast complete devastation of the thermocoagulated cortical tissues without subsequent postoperative irritation, the authors suggest thermocoagulation as a method of treatment for cortical epilepsy when excision of cortex is indicated

GEORGE B HASSIN

HYPOTHERMIA IN HYPOTHALAMIC LESIONS C DAVISON and N E SELBY, Arch Neurol & Psychiat **33** 570, 1935

A man aged 31 presented adiposogenital dystrophy combined with polydipsia, polyuria and prolonged hypothermia The average temperature for three months was 93 F, the minimum (rectal) was 90.7 F, the maximum, 96.6 F Immersion in a hot water bath (108 F) caused a rise in the oral temperature to 100.1 F, immersion in a cold bath, to from 93.2 to 94.2 F Necropsy revealed a hemorrhagic tumor (angioma) between the floor of the third ventricle and the sella turcica It extended from the optic nerve to the mamillary bodies, enclosed the chiasm and optic tracts and destroyed, among other structures, many hypothalamic nuclei but spared the pituitary body The nuclei of the tuber cinereum were destroyed completely, while the vegetative nuclei in the region of the mamillary body were only partly destroyed The partial destruction of the paraventricular and supra-optic nuclei is thought to have been the cause of the slight polyuria and polydipsia

GEORGE B HASSIN

THE EFFECT OF HYPOTHALAMIC LESIONS AND STIMULATION OF THE AUTONOMIC NERVOUS SYSTEM ON CARBOHYDRATE METABOLISM L DAVIS, D CLEVELAND and W R INGRAM, Arch Neurol & Psychiat **33** 592, 1935

The authors studied carbohydrate metabolism in cats in which they had damaged the pancreas, hypothalamus and sympathetic nerves They used the Horsley-Clarke stereotaxic instrument to make lesions in the hypothalamic region with precision Removal of the pancreas in hypophysectomized cats does not produce hyperglycemia or glycosuria, but in those with a lesion of the tuber cinereum it was followed by pancreatic diabetes In a series of cats hypothalamic lesions were made first and the pancreas was removed some weeks later Temporary hyperglycemia followed the bilateral, symmetrical destruction or irritation of the hypothalamus, and in such animals pancreatectomy also caused temporary hyperglycemia followed by normal metabolism of sugar Stimulation of the superior cervical sympathetic ganglion or of the stellate ganglion in cats produced hyperglycemia and glycosuria, which were not present in animals in which the splanchnic nerves had been removed

GEORGE B HASSIN

CORTICOSPINAL FIBERS ARISING IN THE PREMOTOR AREA OF THE MONKEY E C HOFF, Arch Neurol & Psychiat **33** 687, 1935

By studying the degeneration of terminal buttons (*boutons terminaux*) in the spinal cord following experimental lesions of the premotor area in monkeys Hoff could demonstrate that this area gives rise to a separate system of corticospinal fibers The degeneration of the terminal buttons in the spinal cord is bilateral, is usually limited to the base of the dorsal horns, and is predominantly in the contralateral side One group of the corticospinal fibers passes superficially from the premotor into the motor area Hoff has thus furnished histologic proof that, in addition to the motor area, there is a premotor area with a separate group of corticospinal fibers

GEORGE B HASSIN

CORTICOSPINAL FIBERS ARISING IN THE PREMOTOR AREA OF THE MONKEY M A KENNARD, Arch Neurol & Psychiat **33** 698, 1935

Kennard studied with the method of Marchi the system of so-called nonpyramidal corticospinal fibers These originate in the so-called premotor area (Brod-

mann's area 6), ablation of which in monkeys invariably caused descending degeneration of these fibers. Their course is along with the pyramidal tracts to the lower lumbar levels of the spinal cord, contralaterally and ipsilaterally. The degeneration was especially heavy after extirpation of both the motor and premotor areas, but no degenerative fibers were found in the ventral columns after destruction of the premotor area alone.

GEORGE B. HASSIN

THE HEALING OF ARTIFICIAL DEFECTS OF THE DUODENAL MUCOSA II W. FLORIA and H. E. HARDING, J. Path & Bact 40 211, 1935

Brunner's glands are found to regenerate, but on regeneration they occupy for the most part the region of the crypts of Lieberkuhn instead of their usual submucosal site. The duodenal mucosa gives rise to the superficial type of gastric epithelium, which probably arises from Brunner's glands. The possible significance of this is mentioned.

FROM THE AUTHORS' SUMMARY

THE PITUITARY GLAND IN ADDISON'S DISEASE A. C. CROOKI and D. S. RUSSELL, J. Path & Bact 40 255, 1935

A reduction in the number of the basophil cells is considered to be a constant change and the most significant change in the other ductless glands following destruction of the adrenal cortex in Addison's disease. It is suggested that this reduction is the cause of the low blood pressure and possibly of the hypoglycemia in this disease.

HEMOGLOBINOCHOLIA IN TOXIC CONDITIONS R. MUIR and J. F. HEGGIE, J. Path & Bact 40 335, 1935

After administration of various poisons to rabbits hemoglobin in small amounts can be detected in the bile of the gallbladder. This occurs both with poisons which lead to hemoglobinemia and with those which have not this effect. The hemoglobin has been detected only by the Kastle-Meyer reaction, but when some time has elapsed after the death of the animal hemoglobin may be present in such amount as to give distinct spectroscopic bands. This is apparently the result of the action of the bile salts on the erythrocytes within the vessels of the gallbladder mucosa. In cases in which hemoglobin has been found in the bile in the gallbladder it has been detectable also in that of the common bile ducts, its origin is, at least in part, hepatic. These results with the negative results in pure hemoglobinemia indicate that the hemoglobinocholia is due to damage of the liver with escape of a few erythrocytes into the bile capillaries. Such damage in varying degree may be found in phosphorus poisoning but has not been demonstrated in other conditions in which hemoglobin occurs in the bile.

FROM THE AUTHORS' SUMMARY

AUER'S BODIES IN MONOCYTIC LEUKEMIA J. C. HAWKINS, J. Path & Bact 40 365, 1935

The significance of these bodies remains unexplained. They appear to be commoner in the more immature cells and may be due to some rare abnormality in cell metabolism peculiar to leukemic cells. They are not pathognomonic of any type but are of doubtful occurrence in lymphocytic leukemia. It is possible that further investigation of the bodies may link certain cases of leukemia which at present appear not to be closely related.

CULTURES OF LEUKOCYTES IN VITRO G. D. BIFONOVSKI, Arch. internat. de med. exper. 9 367, 1934

In tissue cultures of human blood from a single clot variations in the type of leukocytes predominating commonly occur. If transplanted frequently the cultures

can be kept alive for as long as three months. In cultures of blood from persons with active tuberculosis there is rapid formation of leukocytes, which die sooner than those from normal blood. Tuberculin added in small amounts to cultures destroys the cells of blood from tuberculous patients but shows little or no effect on normal blood cells. Cultures of leukocytes inoculated with virulent tubercle bacilli are protected, to some extent, by a previous inoculation with BCG. Cultures of leukocytes from patients with scarlet fever are also characteristic, showing an increase in eosinophils and many dead cells after forty-eight hours of cultivation. There is evidence that *Bacillus coli* may be transformed into *Bacillus typhosus* by cultivation in symbiosis with human cells. The effect of various mineral waters on cultures of leukocytes is being investigated.

RALPH FULLER

RELATION OF HEALTH TO SOLAR ERUPTIONS. TRAUTE and B. DULL, *Virchows Arch f path Anat* **293** 272, 1935

Traute and Dull review the literature dealing with the relation of human disease and death to meteorological conditions. They criticize the statistical methods used by medical writers in the analysis of meteorological data and the deductions drawn therefrom; they claim that medical writers are not sufficiently familiar with the newer knowledge of geophysics and astrophysics. Such correlation as has been found between weather conditions and disease and death is not proof of the dependence of the latter on the former; both are coordinate manifestations of solar phenomena. For the medical reader a discussion is presented of electronic invasion of the earth's troposphere during the sudden eruptions that occur in the so-called M regions of the sun's surface. The commonest manifestation of such electronic invasion is a disturbance of the earth's magnetic field. Thirty-six thousand deaths occurring in Copenhagen in the five year period from 1928 to 1932 and four thousand in Zurich were analyzed with reference to these solar phenomena. A regularly cyclic heaping of deaths every twenty-seven days was observed. From the analysis of the data it was possible to predict the time after periods of solar activity when deaths in various disease groups would increase. The effects of solar activity on the human organism are ascribed to the action of rays of characteristic wavelength emitted by the sun during periods of eruption.

O. T. SCHULTZ

FORMATION OF BILIRUBIN IN TISSUE CULTURES OF SPLEEN. S. SUMEGI, M. CSABA and E. VON BALOGH, *Virchows Arch f path Anat* **293** 320, 1934

Previous work had led to the conclusion that the formation of bilirubin in tissue cultures of spleen is the result of vital activity of cells of the reticulo-endothelial system and not of an extracellular ferment as has been held by many. Further work on tissue cultures confirmed the authors' previous findings. Potassium cyanide, alterations in hydrogen ion concentration and toxins influenced the formation of bilirubin adversely only when they interfered with cellular activity and growth. Tissue of a spleen that had been subjected to reticulo-endothelial blockade before explantation grew in culture, but bilirubin formation was diminished because of the decreased vital activity of the blockaded cells. Bilirubin was not formed in sterile mixtures of blood and cerebrospinal fluid, contrary to others' findings which have been interpreted as evidence of extracellular ferment activity.

O. T. SCHULTZ

EFFECT OF IONIZED AIR ON THE LEUKOCYTIC BLOOD PICTURE OF GUINEA-PIGS. W. N. NEKLUDOW ET AL., *Virchows Arch f path Anat* **293** 438, 1934

An apparatus was devised by means of which positively or negatively ionized air at a uniform temperature could be delivered to a chamber in which guinea-pigs were kept. Short exposure to negatively or positively ionized air caused temporary leukopenia, monocytosis of short duration and transient increase in pseudo-eosin-

ophils Prolonged exposure to negatively ionized air caused leukocytosis, chronic relative monocytosis, which is ascribed to activity of the reticulo-endothelial system, and decrease in the percentage of lymphocytes

O T SCHULTZ

BLUE SCLERA J HIRSCHMANN, *Ztschr f klin Med* **126** 718 1934

In four of six families with blue sclera there was defective development of bone, cartilage, ligaments, connective tissue and hematopoietic and lymphatic organs Dominant inheritance of blue sclera in the male members of the family was shown in a pedigree of five generations

FROM THE AUTHORS SUMMARY

Pathologic Anatomy

LOCALIZATION OF CARDIAC INFARCTS ACCORDING TO COMPONENT VENTRICULAR MUSCLES J S ROBB J G F HISS and R C ROBB, *Am Heart J* **10** 287, 1935

Certain coronary branches are "end arteries" to the individual ventricular muscle bands If such end arteries are ligated the resulting infarcts affect the electrocardiogram consistently Elimination of either or both of the superficial muscles alters the blood pressure little or not at all Elimination of either of the deep muscles causes a marked fall in blood pressure, and if the lesion is large death is immediate These results cannot be explained according to present-day conceptions of the physiology of conduction

FROM THE AUTHORS' CONCLUSIONS

PATHOLOGY OF CORONARY SCLEROSIS T LEARY, *Am Heart J* **10** 328, 1935

From this study of the lesions of coronary sclerosis it is evident that the pathology of the disease as given in most textbooks needs to be rewritten The pathology in these works is based on cases of coronary sclerosis in which the patients were hospitalized and in which secondary changes, including infarction with associated inflammatory reactions, tended to obscure the picture The material for the present investigation came from patients with early lesions or from patients who literally "dropped dead" This permitted study of the primary process uncomplicated by secondary reactions These studies demonstrated that the standard lesion in coronary sclerosis is atherosclerosis, that the lesions arise from the entrance of lipoids into the subendothelial layer of the intima and from their phagocytosis by cells referred to herein as lipid cells In the young the presence of these lipid cells stimulates growth of fibrous tissue As a result, the standard picture in coronary disease in the young is fibrosis with narrowing of the lumen of the artery Death is usually due to thrombosis In the old the presence of lipid cells does not stimulate fibrosis The cells accumulate in large masses, the nutrition of which becomes inadequate Necrosis and autolysis result in liquefaction of the cell masses, and atheromatous "abscesses" result Death is usually due to the rupture of an atheromatous "abscess" into the lumen It is possible to reproduce the lesions of coronary sclerosis in experimental animals by feeding the lipid cholesterol, which makes up most of the lipid content of atherosclerotic lesions The lesions, natural and experimental, are primarily intimal and lipid Lesions of the elastica and media are secondary Inflammatory reactions are late phenomena following necrosis and are not an essential part of the picture Atherosclerosis is a disease due to disturbances in the cholesterol metabolism and belongs with the other metabolic diseases diabetes (carbohydrates), gout (purines) and obesity (fats)

FROM THE AUTHORS SUMMARY

ACUTE EOSINOPHILIC LEUKEMIA D J STEPHENS, *Am J M Sc* **189** 387, 1935

A patient with acute eosinophilic leukemia is described The clinical and pathologic features of the disease are similar to those observed in other types of

acute leukemia except for the type and maturity of the affected cell group. Evidence of extramedullary production of eosinophils is presented (a high proportion of myelocytes to adult eosinophils and large numbers of immature mononuclear cells in the spleen, lymph nodes and portal spaces of the liver). Lack of adequate neutrophilic myelopoiesis was reflected in the scarcity of neutrophilic granulocytes in the necrotizing inflammatory exudates, which were composed chiefly of adult eosinophils.

FROM THE AUTHOR'S SUMMARY

THE HEMOPOIETIC TISSUES IN HEMOPHILIA R P CUSTER and E B KRUMBHAR, *Am J M Sc* **189** 620, 1935

Three fatal cases of hemophilia with necropsy are reported in one death occurred from uncomplicated hemorrhage, in one from appendicitis and hemorrhage, and in one from fulminating pneumonia. The hematopoietic tissues all showed normal regenerative ability, in the first two cases this was predominantly erythroblastic and in the third it was leukoblastic. All three showed a marked increase of megakaryoblasts and megakaryocytes in the bone marrow, indicating a relationship of the blood platelets to the hemophilic process. J H Wright's observation of platelet formation in the sinusoids of the bone marrow from intruding pseudopods of megakaryocyte cytoplasm was supported by the present findings.

FROM THE AUTHORS' SUMMARY

ETIOLOGIC AND PATHOLOGIC FACTORS IN POLYCYTHEMIA VERA P REZNIKOFF, N C FOOT and J M BETHEA, *Am J M Sc* **189** 753, 1935

Of 134 patients suffering from polycythemia vera about 48 per cent were Jews born in eastern Europe. The records of these patients were obtained from six institutions in which the average incidence of members of this racial and national group was under 10 per cent. Seven specimens of bone marrow from patients with polycythemia vera showed distinct capillary thickening, probably fibrosis, and 6 of these showed in addition marked subintimal and adventitial fibrosis of the subarteriolar capillaries, arterioles and arteries. Of 62 control specimens only 3 of 5 specimens of agranulocytic bone marrow showed slight thickening of capillaries, probably due to edema. The rest showed no change from the normal. In the cases of general arteriosclerosis or arteriolosclerosis medial fibrosis was evident. The vascular changes, especially those of the capillaries, of the bone marrow in patients with polycythemia vera suggest the possibility that these lesions may result in anoxemia of the bone marrow with compensatory or excess compensatory polycythemia.

FROM THE AUTHORS' CONCLUSIONS

THE MYOCARDIAL ASCHOFF BODY L GROSS and J C EHRLICH, *Am J Path* **10** 467 and 489, 1934

The authors have investigated the clinical histories and anatomic material in seventy cases of uncomplicated rheumatic fever in which Aschoff bodies were present in the myocardium. They suggest a classification of Aschoff bodies based on the appearance and distribution of the collagen, argentophil fibers, cell cytoplasm and cell nuclei. This classification includes seven types of Aschoff body, which apparently bear some relation to the life cycle of the lesion. Each type is described and is considered to possess sufficient characteristic features to identify it as an Aschoff body specific for rheumatic fever.

It appears that the Aschoff body passes through three stages in development. The earliest phases, represented by the small cell coronal body and the reticular body, have been found to occur up to the fourth week after the onset of the illness. The middle phases, represented by the large cell coronal, the syncytial coronal, the mosaic and the large irregular cell polarized Aschoff body, have been found to occur between the fourth and the thirteenth week after the onset of the

illness The late phases are represented by polarized Aschoff bodies, which occur from the ninth to the sixteenth week after the onset of the illness, and subsequently by fibrillar Aschoff bodies, which occur after the thirteenth week The earliest specific lesions are apparently influenced in their response by the reactivity of the tissue, depending on whether there has or has not been a previous attack of rheumatic fever, and also by the state of the collagen present in the interstices between the myocardial bundles As a consequence, the evolution of these lesions may follow one of two main courses, determined by the initial lesion The latter may occur in the form of the reticular or of the small cell coronal Aschoff body The final phases of the life of the Aschoff body are common to both main courses Dividing the material into four groups representing different clinical courses, there appears to be some change both in the incidence of the types of Aschoff bodies in the myocardium and in their localization The findings reported here, however, can by no means be considered as furnishing sufficient statistical evidence on which to base final conclusions That the tempo of the life cycle may be considerably faster or slower than has been described in this report seems probable Some of the stages in the life cycle may be absent in some cases, abbreviated in others, or, indeed, may appear in the reverse order from that which we have suggested These facts can be determined with greater accuracy only after a much more extensive series of cases has been examined and, in the last analysis, must await confirmation by the transmission of this disease to animals, attempts at which have thus far been unsuccessful It is hoped that further studies will be made in order that some of these interesting relations may be placed on a firmer footing

FROM THE AUTHORS' SUMMARIES

THE PARATHYROID IN HYPERPARATHYROIDISM B CASTILMAN and T B MAL-
LORY, *Am J Path* **11** 1, 1935

The pathologic findings in the parathyroid glands in hyperparathyroidism may be divided sharply into two types hyperplasia and neoplasia The hyperplasia is characterized by diffuse uniform involvement of all the glandular tissue It occurs, however, in two forms a water-clear type and a much rarer chief cell type A localized tumor of a single gland, part of a gland or rarely parts of two glands is more logically to be regarded as neoplasia A roughly quantitative relation between the size of the enlarged glands and the degree of hyperfunction exists The histology of parathyroid tumors provides confirmatory evidence for the monophyletic theory of the origin of the various cell types Glycogen, albeit in minute amounts, is always present in functioning parathyroid tissue, and the concept of the oxyphilic cell as an inactive involution product receives support from a study of the adenomas

FROM THE AUTHORS' CONCLUSIONS

HEPATIC INFARCTION H LUND, H L STEWART and M M LIEBER, *Am J Path*
11 157, 1935

Seven cases are described which illustrate the red, the pale and the organizing phases of the hepatic infarct An instance of what is possibly a healed infarct is described also Twenty cases of hepatic infarction reported in the literature are summarized

LESIONS OF THE CORONARY ARTERIES IN RHEUMATIC FEVER L GROSS, M A
KUGEL and E Z EPSTEIN, *Am J Path* **11** 253, 1935

A description has been presented of lesions in the main coronary arteries and their branches occurring in active and inactive rheumatic fever, together with a statistical indication of the frequency of their occurrence, the sites of predilection and a comparison of the findings with those in normal controls The lesions in the myocardial branches have been classified under a number of different headings Under four of these the lesions are similar to the evolutionary changes corresponding to advancing age found in the normal controls Several of the remaining

lesions are so peculiar in their structure as to suggest that they may be specific. This observation, however, cannot be accepted as final without an extensive search for similar lesions in other diseases affecting the myocardium. A discussion is given of the mechanism concerned in the development of these lesions, of their possible relation to the development of the arteriosclerotic process and of their clinical significance.

FROM THE AUTHORS' SUMMARY

ENDOMETRIOSIS OF THE UMBILICUS C. V. WELLER, *Am J Path* **11** 281, 1935

With over seven hundred cases described in medical literature the umbilicus can no longer be considered a rare location for endometriosis. Smooth muscle is commonly not present in endometriosis of the umbilicus and is not an essential element in these formations. The term "adenomyoma" is altogether inappropriate. The sweat glands, which have been mentioned repeatedly in connection with umbilical endometriosis, have no part in the genesis of the condition. The origin of umbilical endometriosis is explainable only with the greatest difficulty under the theories that it may result from implantation or metastasis but is readily understandable under the various modifications of the serosal theory.

FROM THE AUTHOR'S CONCLUSIONS

THE ECTOPIC DECIDUAL REACTION AND ITS SIGNIFICANCE IN ENDOMETRIOSIS
C. V. WELLER, *Am J Path* **11** 287, 1935

The subserous stromal cells must possess pluripotentiality in differentiation. Under the influence of suitable but dissimilar stimuli either the "cytogenic" stroma of endometriosis or the decidual reaction may result. If the former develops, it in turn may subsequently be induced to become decidual. In the decidual reaction the mesothelial cells do not appear to take any part. They lie apparently unchanged on the masses of the decidual cells. While not properly germane to the subject of this paper, it must be assumed that the surface mesothelial cells likewise possess latent potentiality in differentiation and that from them, particularly when they are entrapped in adhesions or in scar tissue, and again when they are under the stimulus of a hormone, the epithelial elements in endometriosis are derived.

CYSTIC DISEASE OF THE KIDNEYS E. T. BELL, *Am J Path* **11** 373, 1935

Polycystic kidney is found once in about every five hundred necropsies, and in from 5 to 10 per cent of the cases it is unilateral. In the autopsy service of the department of pathology of the University of Minnesota about one third of the cases were observed in infants, the majority of whom were stillborn. The disease is always congenital. There are relatively few persons between infancy and the age of 25 years who show this condition clinically. One may distinguish a surgical type in which the patient presents symptoms and signs referable to one kidney, viz., pain, tumor, hematuria, infection and so on. In the medical type the symptoms are those of acute or chronic renal insufficiency, and the functional disturbances correspond to those of contracted kidneys. The attacks of hematuria are, however, distinctive. Edema is rarely prominent, and cardiac failure is unusual. The systolic blood pressure is 150 mm of mercury or higher in over 50 per cent of the cases that have been reported, and hypertension is somewhat more frequent in advanced than in early stages of the disease. Cardiac hypertrophy often develops but is much less pronounced than in primary hypertension. Retinal changes of the hypertensive type may be found, especially in those with very high blood pressure. Some patients live many years after symptoms have developed. When the renal reserve is low, i. e., in advanced cases, pregnancy causes typical nephritic toxemia, but causes no disturbance when the renal reserve is good. There is abundant evidence that polycystic renal disease has a strong hereditary tendency. The pyelogram is of great diagnostic value when the diagnosis is otherwise difficult. In the new-born group the outstanding structural changes are the

numerous cysts, hypoplasia of the parenchyma, i. e., a great reduction in the number of nephrons, and an excessive amount of interstitial connective tissue. The numerous "glomerular" cysts are interpreted as vestigial structures derived from the first three or four generations of tubules. In the subclinical group there is abundant renal parenchyma between the cysts, while in the clinical group the parenchyma may be reduced to a few small scattered islands. The progressive atrophy of the parenchyma is brought about chiefly by continuous expansion of the cysts. Arterial disease plays a minor role in this process, except in the occasional case in which true primary hypertension is superimposed on the cystic disease. The arteries usually show marked intimal thickening, which is attributed chiefly to disuse atrophy but partly to hypertension. Medial fibrosis in the arteries is explainable on the basis of age. The arterioles show no marked intimal disease except when primary hypertension is a complication. However, they often show marked medial fibrosis. This process is not true arteriosclerosis. Kampmeier's theory of the origin of the cysts is favored. One case is described in which compensatory dilatation of persistent tubules in a hypertensive contracted kidney caused it to resemble the true congenital cystic kidney.

FROM THE AUTHOR'S SUMMARY

SIMPLE SILICOTIC PROCESS OF THE LUNG F. W. SIMSON, *J. Path. & Bact.* **40** 37, 1935

A technic for the construction of models of discrete simple silicotic nodules with their anatomic relations is described. From these and from serial sections of the nodules the following observations have been made. In the slowly developing type of simple silicosis as seen in the lungs of the Witwatersrand miner, Transvaal, South Africa, the discrete nodule is a sharply defined lesion involving a limited area of tissue in relation to the respiratory bronchioles and the proximal part of their continuation, the alveolar ducts. There is no evidence of "beading" of the larger blood vessels and bronchi from proliferative changes in the related lymphoid tissue. The silicotic nodule is composed of a mass of pigmented loose diffuse fibrous tissue and one or more whorled hyaline islets, which are usually situated in the angle formed where a terminal bronchiole divides into two respiratory bronchioles. The latter pass through the peripheral cellular zone of fibrosis surrounding the islets, and at this point usually show definite stenosis of varying degrees. The terminal air passages are accompanied by branches of the pulmonary artery but not by branches of the corresponding vein. Parts of these terminal arteries are incorporated in the fibrosis of the silicotic nodules but show, as a rule, no evidence of stenosis. The alveolar tissue in the immediate neighborhood of the discrete silicotic nodule almost invariably shows well marked emphysema.

FROM THE AUTHOR'S SUMMARY

THE INCIDENCE OF THROMBOSIS AT NECROPSY H. BELA, *Virchows Arch. f. path. Anat.* **292** 629, 1934

The necropsies in the city hospital at Kiel, Germany, for the years 1913 to 1933 were subjected to statistical analysis to determine the correctness of the assertion that the incidence of venous thrombosis has increased since the World War. In 6,581 necropsies thrombosis was encountered 926 times (14.07 per cent). Pulmonary embolism was observed in 972 per cent of the necropsies. Beginning in 1919 there was a steady increase in the number of cases of thrombosis, the maximum being reached in 1928 with a figure $8\frac{1}{2}$ times the average for the years preceding 1919. Since 1928 there has been only a very slight decrease. Thrombosis occurred about equally in the two sexes and was more frequent after 45 years of age. Thrombosis occurred with very great preponderance in the veins of the lower extremity and of the pelvis. Infection was the most constant single factor, with disease of the circulatory system and neoplasia ranking next. In the pulmonary embolism resulting from thrombosis the right lung was involved more often than the left and the lower lobes more often than other parts of the lungs.

O. T. SCHULTZ

ARTERIOSCLEROSIS IN SWEDEN HELGE SJOVALL and GUNNAR WIHMAN, *Acta path et microbiol Scandinav*, supp 20, 1934, p 1

This study is the Swedish contribution to the conference on arteriosclerosis under the auspices of the International Society for Geographic Pathology. The material comprises 1,075 cases from Stockholm and 305 from Lund. Macroscopic and microscopic studies were carried out on the most important arteries. No marked differences were noted between the two groups as concerns severity and frequency of arteriosclerotic changes. In Lund the women showed a somewhat greater incidence of arteriosclerosis than the men, owing perhaps to more strenuous work. The most severe arteriosclerotic changes were found in the abdominal aorta. The arch of the aorta was second in order of frequency and extent of involvement. In the coronary arteries the descending branch of the left coronary artery is most often involved, and men show earlier and more severe changes in this vessel than women. The abdominal arteries showed a comparatively low incidence and slight change, the splenic artery being most severely involved.

An increase in the average weight of the heart was noted in association with the arteriosclerotic process. Fat persons had a more severe type of arteriosclerosis than thin persons. Tuberculous patients of middle and advanced ages showed slight arteriosclerotic changes. Gallstones and arcus senilis were associated with arteriosclerosis of more than average severity. Only mild arterial changes were found with cancer.

Microscopically, the arterioles of the spleen, liver, pancreas and kidneys were particularly examined. Renal arteriolosclerosis was associated with a more severe type of macroscopic arteriosclerosis.

The lipid deposit in the intima was studied in 290 aortas. Lipoidosis appears to develop in a regular order. The intracellular deposits of lipid occur first, next the extracellular deposit in lamellar arrangement, then a mixed form with coarse lipid deposits in the cells, and finally a typical atheroma. The slightest macroscopic change was associated with intracellular lipid deposits.

JACOB KLEIN

Microbiology and Parasitology

GROUP INFECTION AND IMMUNITY DURING A SCARLET FEVER EPIDEMIC IN A BOYS' SCHOOL B ZUGER, *Am J Hyg* 21 588, 1935

An epidemic of 12 cases of scarlet fever in a school population of 325 boys was studied. During the year of the epidemic twice as many cases of acute tonsillitis occurred as during a similar period of the year before when an epidemic of influenza was present. Also there were 6 cases of rheumatic fever as compared with 1 case the year before. Seven strains of *Streptococcus haemolyticus* were isolated from boys with tonsillitis and 3 from healthy carriers. The toxin produced by 1 strain from each of these groups was subjected to neutralization tests. The reactions in both instances were similar to those of *Streptococcus scarlatinae*. The carrier rate in the healthy population during the epidemic varied from 6 to 20 per cent and two months after the epidemic was still 19 per cent. However, the actual numbers of hemolytic streptococci found in the individual cultures two months after the epidemic were much smaller than during the epidemic. The titre of antistreptolysin was more often elevated in the cases of tonsillitis and sore throat without a rash than in the cases of clinical scarlet fever. It was not elevated to any extent in healthy carriers or in the general population exposed to the epidemic. During the two month period of the epidemic half of the boys who had positive reactions to the Dick test at the beginning gave negative reactions toward the end without having contracted clinical scarlet fever.

FROM THE AUTHOR'S SUMMARY

SOFTENING OF THE CASEOUS TUBERCLE AND ITS RESULTS E. R. LONG, J. A. M. A
104 1883, 1935

Tuberculosis in its epidemiological aspects has its source in a specific pathologic phenomenon—softening of the caseous tubercle.

This phenomenon has long been recognized but not commonly identified as a specific pathologic process, distinct from caseation.

The significance of softening of the caseous tubercle for epidemiology lies in the fact that associated with it is an enormous multiplication of tubercle bacilli. The latter are commonly hundreds or thousands of times as numerous in the semiliquid contents of softening caseous nodules as in the necrotic walls of old cavities.

Three types of case (illustrated by reports in this article) may be distinguished in a general way on the basis of the number of bacilli in the softening regions: a chronic type with a moderate concentration of bacilli in the softening lesions, an intermediate type and an acute type with vast numbers of bacilli.

The fundamental nature of the process of softening is still unknown. It is not equivalent to suppuration. Attempts to put it on an allergic basis have not been entirely successful.

The softening tubercle should receive more clinical consideration. Successful treatment of tuberculosis by collapse of the lung owes its favorable outcome as much to the prevention of drainage of liquefying tubercles as to the obliteration of large cavities. Lung collapse improperly applied, particularly with excessive pressure, even when obvious cavities are obliterated, may result unfavorably through expulsion of highly infective liquefying matter into tributary bronchioles. The most appropriate collapse, as far as the softening tubercle is concerned, is that which stops motion of the lung and partially or completely obliterates the small bronchiolar outlets from the liquefying masses.

FROM THE AUTHOR'S SUMMARY

TOXIC SERUM EXTRACTS OF HEMOLYTIC STREPTOCOCCI J. T. WHEID, J. Exper. Med.
61 473, 1935

The same growth of the hemolytic streptococcus may be subjected to extraction six times in two days with untreated inactivated serum with no loss in potency of the later extracts if it is kept frozen solid during the night between the extractions. The serum-extracted toxins of the hemolytic streptococcus can be preserved without deterioration for at least six months if kept frozen solid. No toxins stronger than those containing 10 units per cubic centimeter for mice have been prepared. Reasons for thinking that this is due to saturation of the serum with the toxin at this point are given. Half saturation with ammonium sulphate precipitates practically all of the hemotoxin in a preparation. Serum extracts were made from strains of the hemolytic streptococcus other than the Gav strain, and attempts were made to correlate the virulence and the production of toxin from each strain. No such correlation could be established. The principal pathologic observation in mice inoculated with the serum-extracted toxin of the streptococcus is a marked degeneration of the tubular epithelium of the kidney.

FROM THE AUTHOR'S SUMMARY

SURVIVAL OF ENCEPHALITIS VIRUS (ST. LOUIS TYPE) IN ANOPHELES QUADRIMACULATUS L. T. WEBSTER, A. D. CLOW and J. H. BAUER, J. Exper. Med.
61 479, 1935

Mosquitoes of this species, fed on mice in the blood stream of which encephalitis virus (St. Louis type) is present, take up and retain the virus for the duration of their lives. The titer of the virus in the mosquitoes four hours after they have become engorged from feeding on mice with a maximum infection of the blood stream represents about 10,000 lethal mouse intracerebral doses per mosquito.

This titer drops during the following two weeks to about 100 lethal doses per mosquito, but from the third week to the death of the mosquito it usually increases to approximately the original level and remains there. The titer of the virus in mosquitoes which have become engorged on mouse blood containing smaller quantities of virus exhibits the same drop and subsequent rise to the original level. The virus-containing mosquitoes did not infect mice or monkeys by biting.

FROM THE AUTHORS' CONCLUSIONS

HEMOLYTIC STREPTOCOCCUS OF HUMAN ORIGIN H. K. WARD and C. LYONS,
J. Exper. Med. **61** 515 and 531, 1935

Four common variants of the hemolytic streptococcus of human origin have been described. These have been designated the F, M, attenuated M, and C variants. Only the F and M variants have been isolated from the blood stream in streptococcic infections. Only the M has any primary virulence for the mouse. Both these variants resist phagocytosis in human blood under suitable conditions, and this appears to be a reliable test for human virulence. The attenuated M variant, found only in laboratory cultures, has a capsule as well developed as that of the virulent variant, and yet does not resist phagocytosis. The C variant has no capsule and is readily phagocytosed. It appears to correspond to the avirulent variant in other species. An attempt has been made to correlate these four variants with those already described in the literature. The application of the findings to the problem of virulence has been discussed.

An antiserum which specifically protects mice against a virulent culture (M variant) of the hemolytic streptococcus contains specific opsonin. Phagocytosis of the organisms can be observed in the peritoneum of the protected mouse. An antiserum prepared by injecting the living M variant into an animal specifically opsonizes both the F and M variant of the strain. Evidence is presented which indicates the probable identity of the specific opsonin and the anti-M precipitin of Lancefield. Agglutination appears to be dependent on a different antibody. It is possible to type the hemolytic streptococci by means of specific opsonins, and the opsonic method has certain advantages over agglutination, precipitation and mouse protection tests. It is evident from what little has been done that there are many types. The serum of infants contains no opsonin for the virulent hemolytic streptococcus, but the serum of adults may contain specific opsonins for certain strains. Inasmuch as no opsonins were demonstrable in two polyvalent antibacterial serums the possibilities of therapeutic transfusion are discussed.

FROM AUTHORS' CONCLUSIONS

ISOLATED OUTBREAK OF EPIDEMIC MENINGITIS G. RAKE, *J. Exper. Med.* **61** 545
1935

The investigation of this isolated epidemic of meningococcic meningitis at a camp of the Civilian Conservation Corps gave an opportunity to examine the state of carrier in contacts carrying what were presumably virulent epidemic strains of the organism. With the aid of Miller's technic for enhancement of the demonstrable virulence of meningococci for mice it proved possible to test the virulence of the strains isolated from carriers at Camp Rusk. These results were consistent despite the interval of from three to four weeks between the isolation of the strains and the titrations of their virulence. Strains of type I were found to have high virulence, while the virulence of strains of type II was moderately high but definitely less than that of strains of type I, and atypical strains and strains of *Neisseria catarrhalis* isolated from carriers showed very low virulence. The question of the precise nature of the state of carrier was investigated. No evidence has been obtained yet as to the existence of a relationship between pharyngitis, coryza or disease of the upper respiratory tract and the presence and degree of the state of carrier. This is unlike the situation with regard to carriers of pneumococcus. On the other hand, it has proved possible to demonstrate reactions

within the body to the meningococci in the nasopharynx, consisting of the formation of agglutinins and protective antibodies in the blood serum, 32.3 per cent of the serums of carriers of type I and 60 per cent of those of carriers of type II showed moderate or good agglutinins for the homologous organisms and 80 per cent of the serums of carriers of type I and 40 per cent of those of carriers of type II showed moderate or good protective antibodies against virulent homologous strains. No idea could be obtained as to the relationship of the presence or absence or of the degree of the serologic reaction to the duration of the carrier state.

FROM THE AUTHOR'S SUMMARY

ENCEPHALOMYELITIS WITH MYELIN DESTRUCTION EXPERIMENTALLY PRODUCED IN MONKEYS T. M. RIVERS and F. F. SCHWINTKER, *J. Exper. Med.* **61** 689, 1935

Repeated intramuscular injections of aqueous emulsions and alcohol ether extracts of sterile normal rabbit brains in some manner produced pathologic changes accompanied by destruction of myelin in the brains of seven of eight monkeys (*Macacus rhesus*). Eight control monkeys remained well. Cultures from the involved brains remained sterile, and no transmissible agent was demonstrated by means of intracerebral inoculations of emulsions of bits of the brains into monkeys, rabbits, guinea-pigs and white mice.

FROM THE AUTHORS' SUMMARY

RABBIT POX WITH ESPECIAL REFERENCE TO EPIDEMIOLOGICAL FACTORS H. S. N. GREEN, *J. Exper. Med.* **61** 807, 1935

A devastating epidemic of rabbit pox in a breeding colony was studied with especial reference to factors of epidemiological significance. The evidence obtained indicated that the epidemic originated among animals inoculated with vaccine virus and that the infection was spread to the breeding colony by caretakers. The epidemic began insidiously with atypical cases of visceral disease followed by typical cases of pox and terminated as a mild cutaneous disease with scattering monosymptomatic disorders of various kinds, difficult to recognize as cases of pox infection. An analysis of data concerning the health and functional efficiency of the population and the immunity of exposed animals showed that the epidemic of rabbit pox was the terminal event in a series of progressive disorders which began fully a month before the first case of pox occurred. In like manner, the terminal decrease in the severity of the disease and the eventual termination of the epidemic appeared to be referable to an improvement in the condition of the population rather than to a specific immunity acquired by exposure to the infection.

FROM THE AUTHOR'S SUMMARY

MULTIPLICATION OF PSEUDORABIES VIRUS IN THE TESTICLE TISSUE OF IMMUNIZED GUINEA-PIGS E. TRAUB, *J. Exper. Med.* **61** 833, 1935

Pseudorabies virus was cultivated *in vitro* in washed testicle tissue from immune guinea-pigs, and evidence was thus procured that the testicle cells themselves had not become immune to pseudorabies. The rate of multiplication of the virus was considerably greater in cultures made with normal guinea-pig testis than in cultures made with immune testis. The reason for this may be that even by repeated washing the immune tissue could not be completely freed from fluid antibodies, and that the remaining antibodies somewhat inhibited the multiplication of the virus.

FROM THE AUTHOR'S SUMMARY

"ROUGHNESS" IN STREPTOCOCCUS CULTURES FROM ENDOCARDITIS R. TUNNICLIFF and C. WOOLSEY, *J. Infect. Dis.* **56** 116, 1935

Eighty-one per cent of the streptococcus cultures from the blood of patients with subacute bacterial endocarditis belonged to the *Streptococcus viridans* group.

No one form of colony was demonstrated in these strains. Eighty-four per cent of the cultures showed signs of "roughness" either morphologically or colonially or both. The observations suggest that the rough element of streptococcus cultures may be an essential factor in the production of endocarditis.

FROM THE AUTHORS' SUMMARY

BACILLARY DYSENTERY IN INFANTS AND CHILDREN G. A. DENISON and G. DE HOLL, *J. Infect. Dis.* **56** 124, 1935

The acute diarrheas of infancy should primarily be divided into (1) gastro-intestinal infections almost invariably due to dysentery bacilli and (2) gastro-intestinal disturbances of function from numerous causes. Before present classifications can be improved, bacillary dysentery must be completely removed from the other types. Infectious diarrheas are bacillary dysentery and should be so designated. Loose clinical terms are not used with uniformity and are confusing. Diagnosis should be supported by examination of the stools microscopically for pus and chemically for blood. The finding of these, with a few very obvious exceptions, always means bacillary dysentery. More reliable data are needed, however, as to the frequency with which blood and pus may be absent in the course of mild dysenteric infections. In investigative work equal consideration must be given clinical, physiologic and bacteriologic phases. In the course of a study of 35 cases of infectious diarrhea in children and infants 159 cultures showing fermentations characteristic of dysentery bacilli were isolated, 142 of these were tested with anti-serums for stock strains and 116 found agglutinable. Of 72 agglutinable cultures tested by absorption of agglutinins in antisera 53 were identified by complete and 15 by partial absorption. The isolated strains corresponded to Y (Hiss), Mt. Desert, WX, V and Sonne. An isolated strain (1-6) was identified by agglutinin absorption with 15 strains from 7 patients. Its antiserum was not appreciably affected by stock strains. It appears to be a member of the Flexner group though not identical with any of the 15 stock strains studied. Dysentery bacilli (Flexner group and Sonne) were isolated from 26 patients with infectious diarrhea (74 per cent). From each of 2 patients 2 different strains were recovered. Dysentery bacilli were recovered from 63 per cent of the stools cultured during the first five days of illness. After the fifth day the chances for recovering the organisms rapidly diminished even though the majority of the stools continued to show blood and pus for fifteen days longer.

FROM THE AUTHORS' SUMMARY,

EFFECT OF SPLENECTOMY ON A LATENT INFECTION, *Eperythrozoon coccoides*, IN WHITE MICE J. MARMORSTON, *J. Infect. Dis.* **56** 142, 1935

In many strains of white mice splenectomy is followed by the appearance of ringlike piroplasmic bodies (*Eperythrozoon coccoides*) on the red cells and in the serum of the peripheral blood. The infection is latent in these strains of mice. The bodies appear after an average incubation period of from six to seven days, and the average period during which the infection is observed is twenty-three and a half days. The height of the infection is reached about the eleventh day. In 15 per cent of the instances *Bartonella muris* is associated with *E. coccoides*, but the *Bartonella* infection appears toward the end of or after the disappearance of the *Eperythrozoon* infection. Suckling mice of noncarrier stock are not infected with *E. coccoides*, and splenectomy in these mice is not followed by the appearance of the infection. The injection of blood containing *E. coccoides* into normal suckling mice does not cause manifestations of the infection, nevertheless, subsequent splenectomy in these mice is followed by the appearance of *E. coccoides* in large numbers within from twenty-four to forty-eight hours, and the disease may be transmitted to splenectomized adult white mice of noncarrier stock.

FROM THE AUTHOR'S SUMMARY

TECHNICAL ERRORS IN STUDIES OF BACTERIAL VARIATION W L HOIMAN and
A E CARSON, J Infect Dis 56 165, 1935

Studies in metamorphoses of bacteria should not disregard the well established principles of pure cultures, and all the various sources of error should be adequately eliminated before far reaching theories are elaborated in this field. Analogies are often dangerous and are particularly so in this instance. It has been shown that complete sterilization of mediums requires careful attention. The spores of some aerobes may withstand 120 C for at least an hour in the autoclave or 100 C for one and one-half hours in the Arnold sterilizer if protected by such a substance as petrolatum when the same spores in broth are destroyed at these temperatures. Many bacteria show morphologic alterations of a temporary nature, and streptococci, spore-bearing aerobic bacilli and various anaerobes are particularly prone to do so. Bacterial association affects the growth of mixed cultures. Streptococci were found capable of inhibiting the growth of spore-bearing aerobic bacilli. These bacilli, moreover, could retain in their colonies streptococci or other bacteria which may readily be overlooked. Inoculation of animals in such studies brings with it a number of chances for contaminating the cultures used. Extraneous bacteria may enter the inoculated area from the site on the skin or from other sources such as the respiratory and intestinal tracts in natural infections or agonal or post-mortem invasions. An analysis of Evans' reports on the metamorphoses of streptococci into spore-bearing rods indicates that these chances of error have not been controlled. We have given more probable and simpler explanations for her findings on the bases of mixed cultures, contaminations and other technical errors. There is need today of a more conservative and critical attitude in these problems of bacterial dissociation since there is a growing tendency to accept uncertain evidence as if it were fundamentally established. FROM THE AUTHORS' CONCLUSIONS

DISSOCIATION OF THE TUBERCLE BACILLUS A SAINZ and L COSTE, Presse med
42 1827, 1934

This study of the dissociation of avian, human and bovine tubercle bacilli and of paratubercle bacilli tends to throw doubt on their specificity. The authors suggest that all varieties of the tubercle bacillus may have a common origin and have become specialized after adaptation to different species. FRIED STRIN

INFECTION WITH SPIROCHAETA PALEA THROUGH COHABITATION AND THROUGH THE PLACENTA W SPIEGEL, Ztschr f Immunitätsforsch u exper Therap
83 386, 1934

Mice were infected with spirochetes. After varying intervals they were exposed to mice of the opposite sex. The syphilitic infection was not transferred, and spirochetes were not found in the offspring or in the placentas. The infection had a distinct inhibitory effect on the fertility of the mice. In female rabbits similarly infected, the placentas contained spirochetes but the offspring was free.

I DAVIDSOHN

MUCOID VARIANTS OF BACTERIUM PARATYPHOSUM B T M VOGELSANG, Acta
path et microbiol Scandinav 12 181, 1935

Among the many hundreds of paratyphoid B strains isolated by us, we have found only two examples of the mucoid variant. Though the mucoid form was demonstrated in the bile in both cases a gradual change to the S form occurred in vitro on subculturing the strains in ox bile. This transformation took place more quickly in the ox bile than in the other fluid mediums employed, viz, peptone water and broth. The S forms derived from the mucoid variants showed a deviation from typical S forms in that they did not form slime walls at room temperature but otherwise reacted—as also did the mucoid forms—like typical Bact paratyphosum B to the different biochemical tests. From the serologic aspect the

two mucoid strains were manifestly paratyphoid B strains, having an "O" antigen in common with the S form and containing the same specific and nonspecific "H" receptors as the latter but in smaller amount

FROM THE AUTHOR'S SUMMARY

BRUCELLA INFECTION OF SWINE A THOMSEN, *Acta path et microbiol Scandinav*, supp 21, 1934, p 1

The material on which the studies recorded in this monograph are based was derived from swine involved in a severe but localized outbreak of brucellosis in Denmark in 1929-1932. In the infected herds agglutination and complement fixation with *Brucella suis* were positive in 39 per cent of the boars that were tested. In such boars purulent and necrotic inflammatory processes were often found in the epididymis, testis and seminal vesicles, and as *Brucella suis* was demonstrated in diseased as well as in normal genital organs the boar was an important spreader of the infection. The spread by boars was a conspicuous feature of the epizootic. The primary lesion in the sow consisted of whitish-yellow nodules in the deeper layer of the inner surface of the uterus (miliary brucellosis). *Brucella suis* was isolated from abscesses in various places in the infected swine. The isolation was not difficult. Thomsen concludes that there is no direct relation between swine and bovine brucellosis, and that there are essential differences between the strains of Danish porcine and bovine *Brucella*. Apparently no definite cases of human infection (undulant fever) developed from the swine brucellosis.

Tumors

PRIMARY CARCINOMA OF THE LUNG C F GESCHICKTER and R DENISON, *Am J Cancer* 22 854, 1934

An analysis of sixty cases of primary cancer of the lung indicates that they may be classified into two groups. The more common are the cases of hilar or epidermoid carcinoma, occurring at the center of the lung and arising from the basal cell layer beneath the lining cells of the large bronchi. The rarer cases are those of lobular adenocarcinoma, occurring at the periphery of the lung and arising from the terminal ramifications of the bronchioles. Hilar or epidermoid carcinoma may be graded into three histologic forms. In the least malignant grade, occurring in patients over 50 years, squamous cells predominate. The middle grade shows a proliferation of basal and transitional cells and occurs in patients under 50 years. The most malignant grade shows masses of compact or spindle cells, is referred to as oat cell cancer, and is most common in young adults. Lobular adenocarcinoma is also divided histologically into three forms. The least malignant, most highly differentiated forms are the adenocolumnar and the adenomucoid. The average age of the patient with these forms is 40 years. The most malignant type shows a diffuse proliferation of cuboidal cells. The patients affected are most often under 40 and sometimes 30 years of age.

FROM THE AUTHORS' SUMMARY

THE HISTOLOGIC CLASSIFICATION OF CANCERS OF THE UTERINE CERVIX AND THE RELATION BETWEEN THE GROWTH STRUCTURE AND THE RESULTS OF RADIUM TREATMENT H CHAMBERS, *Am J Cancer* 23 1, 1935

The biopsy material from 728 cases of cancer of the cervix has been examined and 500 cases have been histologically graded, 228 were unsuitable for classification. The method of grading has been based on the extent of cell differentiation and the degree of cell activity, but the general architecture of the growth has also been taken into account. The relationship of the age incidence, the duration of the symptoms and the clinical type of the growth with the histologic structure has been considered. The results of treatment have been recorded with special reference to local cure of the cancer at the primary site in relation to the histologic type, the survival rate has also been recorded for those cases which had been

under observation for more than three years. The highest percentage of local cures has been obtained in cases of the transitional type of squamous cancer grade 3, 73.8 per cent, and in cases of adenocarcinoma, 72.9 per cent, but none of the histologic grades showed a difference of more than 15 per cent in either local cure or in the number of those surviving three years. There is no evidence in this material that the adenocarcinomas are insensitive to radiation.

STUDIES ON THE INTERNAL ORGANS OF MICE PAINTED WITH CARCINOGENIC AGENTS
J. M. TWORT and C. C. TWORT, *Am J Cancer* **23** 52, 1935

"Our studies have revealed to us that carcinogenic, therapeutic and other agents may so interfere with the general metabolism of mice that there is a profound influence, direct or indirect, on tumour development. Conversely, the presence of a tumour may so derange metabolism, especially of young animals, that cause and effect may be easily confused. It would appear that in order to avoid errors in judgment, investigators should have a thorough knowledge of the changes likely to prevail among their animals. The observer should be in a position to discriminate between the direct and the indirect effect of a therapeutic or carcinogenic agent, with in consequence an added value to his experimental results. General debilitation and stimulation are, it would seem, the key processes to bear in mind. It is when these have been as far as possible accurately gauged, and only then, that we are in a position to evaluate changes in specific tissues. The results we have obtained up to the present time were in many respects to be expected. The general outcome of our observations is that 'balance' is the essential in keeping the animal free from malignant disease, while at the same time the latter supervenes preferentially on a healthy tissue. Additional observations will, we hope, further enlighten us as regards some of the factors responsible for this balance."

THE COMPARATIVE PATHOLOGY OF CARCINOMA OF THE PANCREAS, WITH REPORT OF TWO CASES IN MICE. M. SILE, H. F. HOLMES and H. G. WELLS, *Am J Cancer* **23** 81, 1935

A review of the literature indicates that the occurrence of tumors of the pancreas in all species of animals and birds is extremely rare as compared with their common occurrence in man. Among 125,000 mice of the Slive stock examined post mortem, but two primary tumors of the pancreas, both carcinomas and herewith described, have been observed.

FROM THE AUTHORS' SUMMARY

ON THE BEHAVIOR OF THE ROUS TUMOR VIRUS TO FREEZING. J. K. MILLER and H. E. EGGERS, *Am J Cancer* **23** 94, 1935

The filtrate of the Rous tumor was found to maintain its tumor-producing power after rapid freezing and thawing sixty times when these were so conducted as to avoid accompanying oxidation. There was no apparent change in its action, as determined by rate of tumor growth or by tumor morphology, when this procedure was repeated twenty times. With sixty freezings, there was delay in tumor development with marked change in tumor morphology. The filtrable agent of the Rous tumor displays a resistance to freezing and thawing greater than that exhibited by known living agents, such as bacteria or other cellular organisms. To the extent that this throws light on the nature of the tumor-producing agent, it would suggest an unorganized character. However, the possibility that the filtrate may contain organized bodies so minute as to escape the effect of sudden and repeated changes of volume cannot be absolutely precluded.

FROM THE AUTHORS' SUMMARY

EAR EPITHELIOMA IN THE SYMPATHECTOMIZED ALBINO RABBIT. H. B. RANNEY, *Am J Cancer* **23** 98, 1935

The fact that a majority of investigators have found an increased development of tumors after sympathectomy suggests that the sympathetic nervous system may

exert an inhibitory effect on tumor growth. The directness or indirectness of this influence is as yet a matter of conjecture, the familiar hypothesis that the vascular system acts as an intermediary has not as yet been susceptible of experimental proof.

TUMORS AND TUMOR-LIKE CONDITIONS OF THE LYMPHOCYTE, THE MYELOCYTE, THE ERYTHROCYTE AND THE RETICULUM CELL. G. R. CALLENDER, *Am J Path* **10** 443, 1934.

A discussion of the tumors and tumor-like conditions arising from the stem cells of the lymphocytes, the granular leukocyte, the red blood corpuscle and the reticulum cell or monocyte is presented. Callender offers a classification of these conditions based on a study of the cases of the Lymphatic Tumor Registry of the American Association of Pathologists and Bacteriologists. Certain criteria for the differentiation of the conditions are given in explanation or elaboration of the tabular presentation of the classification. Certain evidence is presented that some conditions ordinarily classified as Hodgkin's disease belong to the reticulum cell group as reactive hyperplasias, aleukemic reticulocytomas or reticulum cell sarcomas.

FROM THE AUTHOR'S SUMMARY

EXTRAGENITAL CHORIOCARCINOMA IN A MAN. A. R. KANTROWITZ, *Am J Path* **10** 531, 1934.

Kantrowitz reports the case of a man aged 22 years who had a primary teratoma of the anterior mediastinum containing choriocarcinomatous elements. At autopsy it was found that the tumor had invaded the superior vena cava and that both lungs were studded with nodules. Careful gross examination revealed no metastases in other organs or lymph nodes. The genital tract (testes, vas deferens, seminal vesicles and prostate) showed no nodules. The testicles were sectioned in 2 mm blocks, and slides were made from each block. The slides revealed no nodules. Microscopic examination of the tumor disclosed teratomatous and choriocarcinomatous elements. Only choriocarcinoma was found in the pulmonary metastases. The testes showed no neoplastic elements. Marked interstitial cell hyperplasia of the testes was seen. These observations refute the contention of Prym and Oberndorfer, the latter writing "*dass beim Mann das Chorionepithelom immer mit Keimdrüsengeschwulsten in Zusammenhang stehen muss*" (that in the male the chorioepithelioma must always be connected with tumors of the gonads). The Aschheim-Zondek test was positive in both the urine and the tumor tissue extracts.

MULTIPLE HEMANGIOBLASTOMA OF THE SPINAL CORD WITH SYRINGOMYELIA (LINDAU'S DISEASE). A. WOLF and S. L. WILENS, *Am J Path* **10** 545, 1934.

A case of hemangioblastomas of the spinal cord forming part of Lindau's disease is presented. These intramedullary tumors were associated with syringomyelia and syringobulbia. The other lesions were a cystic cerebellar hemangioblastoma, congenital cysts of the pancreas and kidneys, a benign hypernephroma of the left kidney, an adrenal rest in a retroperitoneal lymph node and three paragangliomas of the left adrenal gland.

FROM THE AUTHORS' SUMMARY

PRIMARY INTRAMEDULLARY NEUROGENIC SARCOMA OF THE ULNA. J. H. PEERS, *Am J Path* **10** 811, 1934.

Solitary intramedullary tumor of the ulna presenting the histologic structure of a perineurial type primarily occurring in bone must be exceedingly rare, the case reported here being apparently the first recorded. Estimate of the biologic character of the tumor is accordingly uncertain, but on histologic evidence it seems, unlike the neurogenic sarcoma of soft tissues, to be a tumor of low grade malignancy.

FROM THE AUTHOR'S SUMMARY

MALIGNANT TUMORS OF THE LARGE INTESTINE L M LARSON and M NORDLAND,
Ann Surg **100** 328, 1934

A series of 210 cases of malignant tumors of the large intestine is reviewed in this study. There was no preponderance of cases in one or the other sex. The highest age incidence was in the fifth, sixth and seventh years. The growths were located with the greatest frequency at the two extremities of the colon. More than half of the tumors were located in the rectum, rectosigmoid or lower sigmoid, and theoretically, at least, could be visualized through the proctoscope or sigmoidoscope. In about half of the cases coming to necropsy the malignant lesion was mechanically resectable by surgical methods, inasmuch as no extension or metastasis was found at autopsy. About a third of the patients presented metastases in the liver or regional glands. Practically every organ in the body was involved in metastases in this series of cases. No significant difference was noted in the incidence of metastases relative to the location of the lesion. Obstruction took place in 81 per cent of the cases. The immediate cause of death was most frequently peritonitis or exhaustion, but associated lesions such as cardiovascular vascular or pulmonary diseases, hypertrophy of the prostate or acute appendicitis contributed to the low resistance of these patients. In sixteen cases polyposis was present in localized or diffuse form, and in each one of these cases the evidence indicated that malignant change took place on a preexisting benign polyp.

FROM THE AUTHORS' SUMMARY

TUMORS OF THE FROG "ROUSSE" J M PIRLOT and M WILSCH, Arch internat
de med exper **9** 341, 1934

There are skin irregularities in the frog which are similar to cutaneous neoplasms. They occur singly and in groups. They are not transferable by grafting. Small subcutaneous cysts, found mostly in the male frog, can be transmitted by means of grafts of the skin. Two cases of adenocarcinoma and three of adenoma of the skin in man were traced to a single species of frog. A myxofibrochondroma could not be transplanted by grafting.

ELIZABETH McBROOM

ARRHENOBLASTOMA H O KILING, Arch f Gynak **157** 410, 1934

Thirty-five cases of arrhenoblastoma of tubular structure have been reported thus far. Among the four cases that Kleine observed was one in which there was a dermoid cystoma in the other ovary. The growth of these tumors began at the hilus. Leydig's interstitial cells were demonstrable in all. The patients exhibited more or less characteristic signs of virilism. In one patient the rete of the other ovary was considerably enlarged but free from tumor cells. The development of a postoperative hyperthyroidism in one of the patients indicates the possibility of a pluriglandular disturbance. The author agrees with R Meyer that the parent tissues of arrhenoblastoma are the rete ovarii, the medullary strands and the so-called extraglandular interstitial cells. These three epithelial formations represent heterosexual cell complexes of the ovary, which are present in small amounts in every ovary. The hypoplasia of the isosexual gonadal parenchyma seems to promote the development of the heterosexual tissues. The observations of other authors (R Meyer) on younger women with arrhenoblastoma, in whom signs of virilism disappeared after extirpation and reappeared in case of relapse, prove a causal connection between these tumors and virilism. Three problems have yet to be solved: (1) the significance of the rete testis and of Leydig's interstitial cells for the development of the secondary male sex characters, (2) the problem whether arrhenoblastoma forms a testicular incretion, and (3) whether there are relations between arrhenoblastoma and the adrenal system.

INTRATRACHEAL THYMOMA A WALDON, Centralbl f allg Path u path Anat
60 308, 1934

In the body of a woman 58 years old, who had had respiratory difficulty for three months prior to death from bronchopneumonia, a soft tumor was found

attached by a pedicle to the front wall of the trachea, 3 cm above the bifurcation. The tumor was 2.5 cm long, 1 to 1.3 cm wide and 1 cm thick, and the pedicle was 6 mm in diameter. In sagittal section the upper half of the growth contained a cyst 1 cm in diameter, tense, with two outpouchings, and filled with blood. The tissue surrounding the cyst was mottled red while that in the lower half of the tumor was yellow. The mediastinal lymph glands were enlarged from simple hyperplasia, and the thymus was replaced by fat. The microscopic observations were as follows. The stalk was covered with ciliated epithelium and contained one central and two lateral connective tissue bands which delimited two tracts. These tracts consisted of thymic reticulum cells, in which were Hassall's corpuscles, and coursed from the tumor, between two cartilage rings, to the extra-tracheal tissue. The ventral upper part of the tumor was covered with ciliated epithelium which suddenly gave way to stratified squamous epithelium from 5 to 8 cells thick. The papillae underlying this epithelium were short and flat in the upper half of the tumor but were well marked in the lower half. The lower half of the back part of the tumor was partially coated with cylindric epithelium which, in some places, sent tubular glandlike outpouchings into the tumor. At the lower pole there was a mixture of cylindric and squamous epithelium irregularly coating the tumor. Internal to the fibroblastic connective tissue layer in the top half of the tumor were a cellular ground-work and regularly distributed Hassall's corpuscles. In the interior of the lower half collagenic fibers surrounded masses of squamous epithelium with centers consisting of cornified cells. These epithelial masses and strands were continuous with the covering of the tumor. The tumor, therefore, in addition to being a rare growth of thymic origin, was transformed into a carcinoma in its lower half.

GEORGE RUKSTINAT

TUMORS OF THE PERIPHERAL NERVOUS SYSTEM H. J. SCHERER, *Virchows Arch f path Anat* 292 479, 1934

Scherer devotes seventy-five pages to a comparative histologic study of a variety of tumors of the peripheral nervous system, including neuroblastoma of the sympathetic nervous system, neuroganglioma, neurinoma, solitary diffuse overgrowths of peripheral nerves and the neurofibromatous tumors of Recklinghausen's disease. The purpose of the study was to determine the neuro-ectodermal or mesenchymal origin of neurinoma. In the ganglioneuromas there were found cellular areas that Scherer terms "neuro-ectodermal germinal centers." Some of these consisted of undifferentiated cells like those of neuroblastoma, in others, differentiation into ganglion cells was evident. Neurofibril formation in ganglioneuroma occurs independently of ganglion cells. Especial importance is attached to the neuro-ectodermal germinal centers of ganglioneuroma because the presence of similar areas in neurinoma, described for the first time by the author, establishes the neuro-ectodermal origin of neurinoma. This tumor is derived from the ectodermal supporting tissue of the nerves. The germinal tissue does not give rise to ganglion cells, and the neurinoma cannot be considered a peripheral glioma. Neurofibromatosis presents a number of problems that are not possible of solution in the present state of knowledge. In Recklinghausen's disease there is an overgrowth of both mesenchymal supporting tissue and neuro-ectodermal supporting tissue. Which tissue is primarily concerned in the overgrowth it is impossible to determine, both may be equally concerned as the result of not at present understood developmental mechanical factors.

O. T. SCHULTZ

DIFFERENTIAL HISTOLOGIC DIAGNOSIS OF INTRACEREBRAL NEURINOMA. H. J. SCHERER, *Virchows Arch f path Anat* 292 554, 1934

In a case of what is termed "rudimentary Recklinghausen's disease," there were a glioma of the pons and gliosis of the optic and olfactory nerves. In the cerebrum beneath the left ventricle were a number of small nodules that with the usual staining methods were held to be neurinomas. More careful examination of these revealed that the younger nodules were composed of fibril-forming

astrocytes and that the older and denser nodules were composed largely of fibrillated glia. The lesions were not neurinomas but astrocytic glioses or gliomas. His findings lead Scherer to define the histologic criteria that should make it possible to distinguish between a central neurinoma and a glioma. The fibrils of the neurinoma are more sharply contoured and have a straighter course than those of glioma. The neurinoma is more sharply demarcated at its periphery from the surrounding brain tissue. The palisade arrangement of the nuclei, which is the most striking feature of neurinoma and is evidence of the organoid character of the tumor, differs from the pseudopalisade arrangement not infrequently seen in a variety of other fibrillated tumors.

O T SCHULTZ

DIFFERENTIAL DIAGNOSIS OF NEUROGENIC TUMORS II J SCHERER, Virchows Arch f path Anat **292** 562, 1934

In a preceding study of tumors of the peripheral nervous system Scherer reached the conclusion that the presence of perivascular areas of cellular undifferentiated tissue establishes the neurogenic origin of neurinoma. He describes two tumors of the mediastinum in the histologic diagnosis of which ganglioneuroma could be excluded but which could not be diagnosed as neurinoma by the usual criteria. One tumor was fibromatous or fibrosarcomatous, the other, glomatous or more frankly sarcomatous. The denser tumor contained perivascular areas of partly differentiated tissue, the other neoplasm, very cellular perivascular areas of undifferentiated tissue. Scherer thinks that the perivascular tissue is neurogenous in origin and establishes a probable neurogenic origin of the tumors.

O T SCHULTZ

TUMOR-LIKE FORMS OF LYMPHOGRANULOMATOSIS A DUDITS, Ztschr f Krebsforsch **40** 229, 1934

The occasional evidences of polymorphism among the cells of the so-called lymphogranulomatoses may at times be significant of actual malignancy inasmuch as such cells may give rise to malignant tumors apparently of the reticulosarcomatous type. Two such cases are described in this article, the first tumor being primary in the intestinal wall, with relations to the mesenteric nodes but possibly originating in an intestinal follicle, and the second being a typical example of the transformation first discussed.

H E EGGERS

THE INHERITANCE OF CANCER J KORBNER, Ztschr f Krebsforsch **40** 271, 1934

In connection with reports on several instances of the familial occurrence of cancer, Korbner points out that many cases of this sort may be equally well explained on a basis of actual transfer, among his series is one in which the theory of infection would meet the stated facts better than that of heredity. In still another described by him, the family had both tuberculous and cancerous antecedents, and here there was a curious separation of the two diseases in different branches of the family. As regards the relations of tuberculosis to cancer, he is of the opinion that as cancer is outstandingly a disease of unimpaired vitality, the previous presence of tuberculosis would react against the development of the former disease. The reverse of this relationship, he believes, is shown by the relation of cancer to longevity, in which there would occur long continuance of unimpaired vital function.

H E EGGERS

Technical

AN ULTRAMICRO TECHNIC FOR PRECIPITIN AND AGGLUTININ REACTIONS C L HUDSON and S MUDD, J Immunol **28** 311, 1935

The capillary tube technic developed by Richards, Bordley and Walker (*J Biol Chem* **101** 179, 1933) for the chemical ultramicro-analysis of minute amounts of

fluid has been adopted for the performance of certain immunologic reactions. Precipitation and agglutination tests have been made with as little as 0.1 cu mm of reacting material. In sensitivity and reliability this technic was found equal to standard macromethods. The apparatus required is neither expensive nor difficult to obtain and the technic is easily mastered. The obvious advantages of the ultramicromethod are the minuteness of the quantity of fluid required and the rapidity with which a determination can be completed. An additional advantage is the opportunity for microscopic observation of the precipitation and agglutination during the course of reaction. The vividness with which the reactions may be seen contributes an appreciation of the character of these phenomena even to an observer experienced with macromethods.

FROM THE AUTHORS' SUMMARY

A SIMPLE DIFFERENTIAL STAIN FOR THE HUMAN HYPOPHYSIS C SPARK, J Lab & Clin Med 20 508, 1935

Fixation—Orth's fluid (Muller's fluid, 9 parts, dilute solution of formaldehyde, 1 part) is superior to fixatives containing mercuric bichloride such as Zenker's or Helly's fluid, which result in a marked brittleness of the tissue. The tissue is fixed for forty-eight hours and washed in running water for twenty-four hours.

Staining—Paraffin sections from 6 to 8 microns thick are brought down to water. The sections are stained as follows:

- 1 Immerse in 0.25 per cent aqueous aniline blue for from sixty to ninety seconds. The basophil cells are stained deep blue, while the rest of the tissue is stained very faint blue.

- 2 Wash in tap water for one-half minute. Excessive washing will remove the dye from the basophils.

- 3 Immerse in Mayer's hematoxylin for ten minutes.

- 4 Wash in tap water for from two to three minutes.

- 5 Immerse in Van Gieson's mixture for from sixty to ninety seconds. The mixture is made up by adding 5 cc of 2 per cent aqueous acid fuchsin to 100 cc of saturated aqueous trinitrophenol. Excessive treatment with Van Gieson's mixture will result in a greenish blue coloration of the basophils.

- 6 Wash in tap water for one minute. Prolonged washing will wash out the Van Gieson stain.

- 7 Transfer sections to 95 per cent alcohol for one minute.

- 8 Dehydrate in absolute alcohol for from two to three minutes.

- 9 Clear in xylene.

By this method the various cellular elements of the human hypophysis are sharply delineated. Nuclear chromatin is stained purplish brown. The beta (basophilic) granules are stained dark blue and appear in sharp contrast to the alpha (acidophilic) granules, which stain olive green. The cytoplasm of the chromophobe cells are light grayish blue. Red blood cells stain bright yellow, which adds considerably to the ease of studying the sections, especially in glands that show congestion of the blood vessels. The colloid in the anterior lobe stains a variety of colors varying from yellowish to purple. The colloid in the posterior lobe stains light blue.

Dense collagenic fibers stain deeply with the acid fuchsin, while loose collagenic connective tissue stains lightly either with aniline blue or with acid fuchsin. The capillary walls stain sharp blue. The stained sections possess a good degree of optical translucency, which was not obtained with several of the published stains for the hypophysis.

Stained sections that are now two years old have not shown any significant degree of fading.

Book Reviews

A Textbook of Biochemistry Edited by Benjamin Harrow, Ph D, Associate Professor of Chemistry, The City College, College of the City of New York, and Carl P Sherwin, M D, Sc D, Dr P H, Member of the Staff of St Vincent's Hospital, New York Cloth Price, \$6 Pp 797, with 52 illustrations Philadelphia W B Saunders Company, 1935

In the preface to this book the editors state "Biochemistry, like several other branches of science, has become so encyclopedic in its scope, that it seems an impossible task for any one individual to write an adequate textbook. It is for this reason that we have asked specialists in the various fields of biochemistry to contribute the chapters constituting this book." This explains the general nature of the book. It has been divided into thirty chapters, written by as many different authors, all professors or investigators in the field of biochemistry.

The book covers a somewhat wider range than the usual textbook of biochemistry. In the first chapter Chambers discusses the action of the living cell. The next six chapters deal with the chemistry of carbohydrates, fats, sterols, proteins, amino-acids and nucleic acids. Chapters 8, 9, 10 and 11, dealing with nutrition, the vitamins, the enzymes and digestion, offer a general and comprehensive discussion of these subjects. The chapter on the biochemistry of bacteria, yeasts and molds by Clutterbuck and Raistrick is of particular interest because it presents one of the new fields for investigation in biochemistry. Carl P Sherwin presents a detailed dissertation on detoxication in chapter 13. Chapters 15, 16, 17 and 18 are discussions of the blood and blood gases, respiration and animal pigments. The oxidation-reduction reactions of cells are described in detail in chapter 19. In this chapter, as in the chapter on immunochemistry, the material presented is highly specialized and might prove somewhat detailed for the beginning student of biochemistry and for the medical student.

The scope of Heidelberger's chapter is well illustrated in his summary, which is also of interest because it indicates the present state of knowledge of the chemistry of antigen-antibody reactions. Heidelberger states "In this chapter the writer has tried to show how chemistry has made steps toward giving more definite meaning to the concepts antigen and antibody and providing a better understanding of the mechanism of the immune reactions in which they participate. The introduction of known chemical groups into the protein molecule, with its consequent sensitive control of specificity, and the recognition of the large part played by polysaccharides in bacterial specificity have served to emphasize the essentially chemical and ultimately minutely determinable basis of biological specificity, and have simplified and clarified relationships and provided powerful aids for further progress. With highly purified antibody close at hand, and with plausible theories as to its formation, the many problems connected with antibodies should be well on their way toward solution. With these newer aids it has been possible to obtain strong evidence of the chemical union of antigen or hapten with antibody in multiple proportions, and to express this union in terms of the laws of classical chemistry. On this foundation there are now accessible new and absolute quantitative methods which should be useful tools in the acquisition of a final complete understanding of immune processes."

The succeeding chapters deal, for the most part, more generally with the metabolism of proteins, carbohydrates, fats and minerals. The chemistry of the skin, muscle, bone, brain and urine are discussed in the latter part of the book, the last chapter being a short discussion of hormones. In general, the articles are well written and interesting. However, as a textbook, the result may be somewhat too detailed for the student of medicine and the beginner in the field of biochemistry. The book might well be used for supplementary reading, for it presents a comprehensive and highly detailed discussion of the various aspects of

biochemistry This specialization necessitates the omission of many fundamental facts essential in a textbook for students The bibliographies at the end of each chapter offer a wealth of material The book is highly recommended for teachers, advanced students and investigators in the field of biochemistry

The Spleen and Resistance By David Perla, M D, Associate Pathologist, Montefiore Hospital, and Jessie Marmorston, M D, Associate in Pathology, Cornell University Medical College Price, \$2 Pp 170 Baltimore Williams & Wilkins Company, 1935

The book begins with a review of the anatomy of the spleen The major elements in its structure are derived from reticular cells or mesenchymal reticulum In infections the structural changes, and presumably also the functions, vary according to the species and the type of infection The large number of macrophages in the spleen indicates its capacity for phagocytosis, but it is not assumed that phagocytosis is the only or the most important factor in resistance From an analysis of the experiments on the formation of antibodies by the spleen it is concluded that splenectomy lowers the power of the body to form antibodies Some of these experiments failed to take into consideration the circumstance that in dogs, mice and rats splenectomy may change certain existing latent infections (those due to Bartonella, Klossiella, eperythrozoon coccoides) into active diseases In these and other types of infection splenectomy depresses natural as well as acquired resistance in many species of animals In many of these diseases the macrophagic tissues of the spleen are involved Splenectomy is followed also by proliferation of macrophages in the lymph nodes, liver, marrow and lungs This proliferation is interpreted as compensatory in nature and due to an activation from splenectomy of mesenchymal reticular cells and their derivatives everywhere in the body The role of the spleen in resistance consequently is not to be ascribed wholly to phagocytosis, because it may be involved in "subtle chemical interrelationships, at present little understood" However, the importance of the spleen in natural and acquired resistance to certain bacterial and protozoan infections is now well established The study of the spleen in latent infections by the authors and others throws a new and helpful light on experiments in splenic physiology These experiments have resulted in contradictions and controversies because they dealt with different species and with carriers of latent infections that induce anemia when the spleen is removed "It becomes extremely questionable, in view of these facts, that the spleen plays the role in hemoglobin formation previously attributed to it on the basis of studies of iron metabolism in splenectomized animals in which the presence of latent infection has not been excluded"

There is a good bibliography, also complete subject and author indexes While this is of minor importance, perhaps, note must be made of the fact that the proper names of micro-organisms are printed sometimes in italics and sometimes in ordinary type and in either case sometimes with and sometimes without the usual initial capitalization The monograph presents well the present understanding of the relation of the spleen to resistance to infection It will be of value in the further study of the spleen in health and disease

Books Received

PRECIS DE THERAPEUTIQUE ET DE PHARMACOLOGIE R Hazard Professeur agrège a la Faculté de Médecine de Paris, Pharmacien des Hôpitaux With a Preface by A Desgrez, Membre de l'Institut et de l'Académie de Médecine Third edition, completely revised Price, stitched, 85 francs, bound, 100 francs Pp 1257, with 34 illustrations Paris Masson & Cie, 1935

ANGINES LYMPHO-MONOCYTAIRES AGRANULOCYTOSE, LEUCÉMIES LEUCOPÉNIQUES J Cabrazes, Professeur a la Faculté de Médecine de Bordeaux, and René Saric, Interne des Hôpitaux de Bordeaux Price, 40 francs Pp 364, with 18 figures Paris Masson & Cie, 1935

This book contains three chapters The first deals with the disease or group of diseases which in the English literature are now perhaps most frequently called infectious mononucleosis The second chapter is devoted to the conditions included under the term agranulocytosis, and the third chapter to aleukemic leukemia and allied diseases Each chapter is a complete monograph by itself, with a select bibliography of original articles and reviews on the subject with which it deals At the end of the book is a general subject index, which is a rather unusual feature in a French publication of this kind The more important literature concerning the diseases under consideration is reviewed carefully, and a number of illustrative cases observed by the authors are reported in detail The book will give the reader a clear and comprehensive summary of the present knowledge and views of the different phases and relations of infectious mononucleosis, agranulocytosis and aleukemic leukemia—all topics of actively growing interest and study at this time

THE SPLEEN AND RESISTANCE David Perla, M D, Associate Pathologist, Montefiore Hospital, and Jessie Marmorston, M D, Associate in Pathology, Cornell University Medical College Price, \$2 Pp 170 Baltimore Williams & Wilkins Company, 1935

EFFECT OF IODINE ON CHOLESTEROL-INDUCED
ATHEROSCLEROSIS

DOROTHY R MEEKER, A B

H D KESTEN, M D

AND

JAMES W JOBLING, M D

NEW YORK

Although iodine has been used empirically for many years in the treatment of arteriosclerosis in man, experimental proof is lacking that it can alter an arteriosclerotic plaque. There is evidence, however, that when administered in organic or inorganic combination to rabbits fed large amounts of cholesterol, iodine can inhibit, at least partially, the production of the characteristic arterial lesions induced by cholesterol. Until now, however, there has been no study of the effect of iodine on fully developed cholesterol-induced atherosclerosis. In view of the protective power of the drug against atherosclerosis induced by cholesterol and with its effect on infectious granuloma in mind, it seemed reasonable that iodine might accelerate the healing and absorption of cholesterol-induced lesions. Were this true, it would suggest another basis for the utilization of iodine in arteriosclerosis in man.

The procedure used in the study of the possible resorptive effect of iodine on the lesions of experimental atherosclerosis was to produce such lesions by adequate feeding of cholesterol to rabbits and then to feed potassium iodide for various periods.

METHOD OF INVESTIGATION

Twenty-five gray chinchilla rabbits, each weighing 2 Kg, were used. According to the technic of Turner¹ each rabbit was fed 1 Gm of cholesterol mixed with its food four times a week for one hundred and five days. This period of time and this dosage have been demonstrated to be ample for the regular production of aortic lesions. The feeding of cholesterol was then stopped, and the animals were divided into three groups as follows: (1) Nine rabbits were killed immediately as controls, (2) eight rabbits were allowed to rest on an ordinary stock diet, two of which were killed one month after the cholesterol was discontinued, three two months after and three three months after, and (3) the remaining eight rabbits were placed

This investigation has been aided by a grant from the Josiah Macy Jr Foundation.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

¹ Turner, K B. *J Exper Med* 58:115, 1933.

on the same stock diet and were fed 0.85 Gm of potassium iodide daily by pipet in the form of 1 cc of a concentrated solution (60 Gm of potassium iodide in each 70 cc of solution). This method of administration proved satisfactory. The rabbits gained weight and remained in good condition. Two of them were killed one month after the cessation of the feeding of cholesterol and the commencement of potassium iodide, three two months after and three three months after. The content of cholesterol in the serum, both the total and free cholesterol, was determined at frequent intervals by the method of Schoenheimer and Sperry.²

When examined at the end of the experimental periods all the rabbits presented vascular lesions of the type due to cholesterol. The aortas of the nine rabbits used as controls, which were killed at the end of one hundred and five days of feeding of cholesterol, presented characteristic intimal plaques, varying considerably in extent and number from a few small lesions in the arch to extensive confluent infiltrations throughout the vessel. Similar lesions were not infrequently present in the cusps of the mitral and aortic valves, the larger pulmonary arteries and the main branches of the aorta. In gross appearance and frequency the lesions in the aortas of rabbits fed cholesterol for one hundred and five days and allowed to rest on a stock diet for from one to three months could not be distinguished from those in the first group used as controls. The aortic lesions of rabbits of the third group, those which received potassium iodide after cessation of the cholesterol, differed in no essential respect from those of the other animals. If anything, the lesions in the aortas of these rabbits were somewhat more extensive than those in the animals which were resting on the stock diet. Nor did microscopic study reveal any difference between the lesions in the animals given potassium iodide and those in the animals which received none. Though there was evidence of continuing fibrosis of the cholesterol-induced plaques after the feeding of cholesterol was stopped, there was no real difference in the amount of fibrosis between lesions in the resting animals and those in rabbits fed potassium iodide (table). There was considerable variation in the degree of atherosclerosis within each group, but this could not be ascribed to lack of absorption of cholesterol, for in every animal the cholesterol content of the serum was well above normal, in all but three cases being more than 700 mg per hundred cubic centimeters of blood, and the content remained high for a considerable, though variable, time after the feeding of cholesterol was stopped. There was, then, no correlation between the extent of sclerosis and the experimental procedure.

COMMENT

The inability to alter fully developed cholesterol-induced arterial lesions with moderately large doses of potassium iodide whereas iodine

² Schoenheimer, R, and Sperry, W M. *J Biol Chem* **106** 745, 1934

Values for Cholesterol of the Serum in Rabbits Fed Cholesterol

Rabbit	Length of Experiment, Days*												Length of time on Potassium Iodide or Resting, Months	Degree of Athero sclerosis						
	42			96			112†			134					149			197		
	Total, Mg per 100 Cc	Ratio Ester/Free	Total, Mg per 100 Cc	Ratio Ester/Free	Total, Mg per 100 Cc	Ratio Ester/Free	Total, Mg per 100 Cc	Ratio Ester/Free	Total, Mg per 100 Cc	Ratio Ester/Free	Total, Mg per 100 Cc	Ratio Ester/Free			Total, Mg per 100 Cc	Ratio Ester/Free	Total, Mg per 100 Cc	Ratio Ester/Free		
							With Potassium Iodide													
205	868	3 32	840	2 03	1,184	1 55	761	1 09									1	++-+		
207	535	3 11	251	1 76	346	1 29	449	1 09									1	+		
202	666	2 66	1,088	3 51	488	2 03	265	2 08	186	1 82							2	++		
203	593	2 87	807	2 40	492	2 64	319	2 04	184	1 92							2	++		
204	542	1 88	1,015	2 80	725	2 44	322	1 87	249	1 77							2	+		
208	402	3 28	874	2 36	611	2 55	718	1 98	465	1 72	93	1 45					3	++		
209	376	2 55	875	2 74	721	1 99	467	1 73	203	1 54	164	1 55					3	++-+		
211	630	2 52	860	2 28	705	2 67	566	2 27	328	1 78	204	2 10					3	++-+		
							Without Potassium Iodide													
206	72	4 15	336	2 57	271	3 11	99	2 00									1	+		
213	177	4 06	840	2 84	842	3 15	475	2 60									1	+		
216	520	3 05	670	3 21	511	2 87	279	2 98	106	2 78							2	+		
217	524	3 40	946	3 36	565	3 01	141	2 86	109	2 76							2	+		
221	801	2 96	920	2 25	880	2 78	403	2 67	264	2 67							2	++-+		
218	689	2 49	1,004	2 60	880	2 89	434	2 68	285	2 48	123	1 98					3	++-+		
219	886	3 12	832	1 20	1,105	1 98	635	1 90	464	1 99	69	2 64					3	++		
222	33	2 88	331	3 09	291	2 68	144	2 20	73	2 76	25	3 03					3	+		

* Feeding of cholesterol was stopped on the one hundred and fifth day
† One week after feeding of cholesterol was stopped

in both organic and inorganic form when given early has the power of preventing the deposition of cholesterol and its esters (Liebig, Seel and Creuzberg, Mori and Shinoi, Turner, and Ungar)³ points to irreversibility of the atherosclerotic process. Even after many months the lesions remain as fibrous plaques⁴ (There is little doubt, however, that the frequent intimal plaques seen in typhoid fever and other febrile disturbances, especially in childhood, are resorbed.)

The distinction which must apparently be made between cholesterol-induced lesions which are developing and those already formed is further emphasized by a study of the cholesterol of the serum. In the normal rabbit the ratio of the amount of cholesterol esters to that of free cholesterol in the blood serum varies between 1.85:1 and 3:1. During the period of feeding cholesterol this ratio is generally not appreciably altered, the amounts both of free cholesterol and of cholesterol esters increasing to the same degree. Liebig,^{3a} Seel and Creuzberg³ and Turner¹ found that administration of iodine with cholesterol resulted in a reduction in the cholesterol content of the serum and in protection against atherosclerosis. (Rosenthal,⁵ however, reported an increase in the amount of cholesterol in the blood and in the degree of atherosclerosis when small amounts of iodine were administered [from 2 to 3 mg of iodine daily], which was in contrast to the findings of Seel and Creuzberg, who also used small doses. The major difficulty in comparing the findings of the several workers in this field is that there is no uniformity of procedure, the amount of cholesterol and of iodine given and the duration of feeding cholesterol, both before and with the administration of iodine, varying from one experiment to another.) It was found in the present study, however, that when iodine was administered after the cessation of feeding of cholesterol there was a progressive decrease in the ratio of the amount of cholesterol esters to free cholesterol as the feeding of potassium iodide continued (table), until after six weeks of administration of potassium iodide the ratio in every case was at or below the lower limit of the normal value, whereas the ratios in the resting animals were significantly higher. This is in contrast to the findings of Rosenthal, namely, that the value for the esters remained high for as long as six weeks when potassium iodide was fed with cholesterol. It was further observed that, although the amount of cholesterol

3 (a) Liebig, H. *Med Klin* **25** 1100, 1929, *Riforma med* **47** 1400, 1931, *Arch f exper Path u Pharmacol* **159** 265, 1931. (b) Mori, K., and Shinoi, K. *Mitt d med Gesellsch zu Tokio* **46** 316, 1932. (c) Seel, H., and Creuzberg, G. *Arch f exper Path u Pharmacol* **161** 674, 1931. (d) Ungar, H. *ibid* **175** 536, 1934. (e) Turner¹.

4 Anitschkow, N., in Cowdry, E. V. *Arteriosclerosis*, New York, The Macmillan Company, 1933, p. 291.

5 Rosenthal, S. R. *Arch Path* **18** 827, 1934.

esters in the blood was rapidly decreasing in the rabbits receiving potassium iodide, the total cholesterol content remained relatively high. Six weeks after the feeding of cholesterol was stopped none of the rabbits given potassium iodide had a normal amount of cholesterol in the blood, whereas that of three of the resting group was again within the normal range. Even at the end of three months, the blood of only one of the three remaining animals given potassium iodide had a normal cholesterol content, while that of all three of the remaining resting animals had reached the normal level. This retardation of the return of the cholesterol of the blood to the normal level when potassium iodide was administered is not in harmony with the findings of Seel and Creuzberg, who reported a more rapid decrease in the amount of cholesterol in the blood of the animals fed iodine after twenty-four days of administration of cholesterol. An explanation of the aforementioned differences is not obvious, although both Rosenthal and Seel and Creuzberg administered smaller doses of iodine.

The relatively rapid decrease in the amount of cholesterol esters in the blood serum of the rabbits receiving potassium iodide implies either storage or destruction of the esters. The presence of large amounts of free cholesterol in the blood serum suggests mobilization of stored cholesterol and favors the hypothesis that the esters were destroyed. The mobilization of stored cholesterol under the influence of potassium iodide, if it occurs, not only would account for the retardation of the return of the cholesterol content of the blood to normal levels but might also explain the somewhat greater degree of atherosclerosis in the rabbits which received potassium iodide.

The experiments of Murata and Kataoka,⁶ Friedland⁷ and Turner and Khayat⁸ pointed to the thyroid gland as having a rôle in the prevention of experimental atherosclerosis in rabbits. Examination of the thyroid glands of the rabbits fed cholesterol for one hundred and five days and then either permitted to rest for from one to three months or fed potassium iodide for the same periods failed to reveal consistent differences. Practically all the glands in both groups were composed predominantly of large follicles, lined by flat epithelium and filled with well stained colloid. There was no correlation between the state of the thyroid gland and the degree of atherosclerosis.

SUMMARY

Potassium iodide fed in large doses for from one to three months to rabbits in which atherosclerosis had previously been induced by pro-

6 Murata, M., and Kataoka, S. *Verhandl. jap. path. Gesellsch.* **8**: 221, 1918.

7 Friedland, I. B. *Ztschr. f. d. ges. exper. Med.* **87**: 683, 1933.

8 Turner, K. B., and Khayat, G. B. *J. Exper. Med.* **58**: 127, 1933.

longed feeding of cholesterol does not influence the rate or nature of the involution of the vascular lesions

Potassium iodide appears to retard the return of the cholesterol content of the blood to normal levels although it markedly depresses the ratio of the amounts of cholesterol esters and free cholesterol. It is suggested that this retardation may be due to mobilization of stored cholesterol from the tissues.

Dr. W. M. Sperry of the Babies Hospital determined the values for cholesterol

EXPERIMENTS RELATIVE TO VACCINATION AGAINST TUBERCULOSIS WITH THE CALMETTE-GUÉRIN BACILLUS (B C G)

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During recent years a great deal of interest has been stimulated in the possibility of increasing the protection of children against tuberculosis by vaccinating them with the Calmette-Guérin bovine strain of the tubercle bacillus (B C G). In most cases the vaccine has been administered orally, but recent efforts have been directed toward the use of the vaccine injected as living organisms subcutaneously. This method of giving the vaccine has been used by Heimbeck¹ and by Park and his associates².

The literature on B C G is extensive, and a complete analysis is not attempted in this paper. A comprehensive digest of the literature that appeared up to 1929 was published by Petroff³. Since 1929 critical reviews have been written by Bouquet,⁴ Kraus⁵ and Lurie⁶.

From these reviews it is evident that there is still lack of agreement in regard to the innocuousness of B C G. Most workers seem to agree that for the most part B C G may be looked on as a strain of the tubercle bacillus which has lost its property of producing progressive tuberculosis. Other experimenters have reported that under certain conditions of cultivation, as in deep peptone broth (Dreyer and Vollum⁷), by the addition of normal rabbit serum (Sasano and Medlar⁸) or by growing the organisms in contact with antiserum (Petroff), the strain may become virulent and produce progressive tuberculosis. Numerous experiments have been reported, some seemingly to prove and some to disprove these contentions. The recent work of Park and his associates seems to show fairly definitely that the inoculation of children subcutaneously with large doses of living B C G carries with it no definite danger.

From the Department of Pathology, the University of Minnesota

1 Heimbeck, J. Arch Int Med **49** 957, 1932

2 Park, W. H., Kereszturi, C., and Mishulow, A. B. J. A. M. A **101** 1619, 1933

3 Petroff, S. A. Am Rev Tuberc **20** 275, 1929

4 Bouquet, A. Am Rev Tuberc **24** 764, 1931

5 Kraus, Rudolf. Am Rev Tuberc **24** 778, 1931

6 Lurie, M. B. J. Exper Med **60** 163, 1934

7 Dreyer, G., and Vollum, R. L. Lancet **1** 9, 1931

8 Sasano, K. T., and Medlar, E. M. Am Rev Tuberc **23** 215, 1931

Calmette⁹ observed more than 400 autopsies performed on children who had been vaccinated but had died of other causes. In none of these children did he note evidence of tuberculous lesions.

There is also failure among experimenters to agree in regard to the value of BCG in developing resistance to subsequent infections with virulent bovine or human strains. Most observers agree, however, that a degree of resistance follows vaccination.

In the experiments reported in this paper an effort was made to study quantitatively immune reactions in experimental tuberculosis. Allergy and resistance were studied as related to each other and as each is related to antibodies, agglutinins, complement-fixing antibodies, opsonins and lysins.

The relation of these reactions to the pathogenesis of pulmonary tuberculosis was observed to see whether in a comparative way helpful conclusions could be drawn concerning the pathogenesis of pulmonary tuberculosis in man.

MATERIALS AND METHODS

The strain of BCG used in the experiments was obtained from Feldman¹⁰ and is the one used by him to produce lesions in rabbits. Feldman obtained this strain from Calmette in January 1930. The organism was cultured by Feldman on glycerin-broth-potato medium until October 1930. Subcultures were made every four weeks. From October 1930 until April 1933 the organism was grown with monthly transfers on Feldman's modified glycerinated egg medium. Since April 1933 this strain has been subcultured each month on agar to which was added 0.2 per cent dextrose, veal infusion and 5 per cent glycerin. It has not come in contact with bile since October 1930. Since coming into my possession it has been kept most of the time at 37 C., and large quantities have been grown.

The virulent bovine strain (Ravenel) used in testing resistance in rabbits was supplied by Lurie¹¹ from the Henry Phipps Institute. Lurie found that when 0.01 mg. of these organisms was injected intravenously into rabbits death from pulmonary and generalized tuberculosis resulted on an average in about forty days. These findings were confirmed in my work. The human strain (H 37) was used in testing resistance in guinea-pigs.

Old tuberculin was used in determining the presence and degree of allergy. One milligram of old tuberculin was injected intracutaneously. The volume injected was 0.1 cc. This amount injected into the skin of the rabbit or guinea-pig produced an excellent wheal. The tuberculin was of such potency that when 0.05 mg. was injected intracutaneously into a person allergic to the tubercle bacillus a strong positive reaction was elicited. Reactions in the rabbits and guinea-pigs were observed forty-eight hours after the injection of the tuberculin. Redness with slight induration was read as +. Extreme redness and induration with necrosis were reported as +++++. The readings ++ and +++ indicated intermediate stages of induration without necrosis. Necrosis in the area of injection was required for a reading of +++++.

9 Calmette, A. *Am Rev Tuberc* **27** 1, 1933.

10 Feldman, W. H. *Am J Path* **8** 755, 1932.

11 Lurie, M. B. Personal communication.

The degrees of development of the microscopic lesions observed in the visceral organs of the animals were recorded in grades of from + to +++++. Grade + represented only an occasional area of tuberculous pneumonia or a small, solitary epithelioid tubercle. Grade +++++ represented many tubercles or areas of tuberculous pneumonia scattered thickly throughout the slides. Grades ++ and +++ indicated intermediate stages.

Rabbits weighing from 3 to 4 pounds (1.3 to 1.8 Kg.) were used in studying safety and resistance and guinea-pigs in studying resistance developed to the human strain.

The rabbits in the regular course of vaccination received four injections of 1 mg. of BCG at intervals of one week. Several rabbits, for comparison of the degrees of allergy and resistance, were given more injections or larger amounts, or both. When it was desired to make animals allergic each rabbit was given one injection subcutaneously of from 1 to 4 mg. of living BCG.

The BCG used for vaccination was ground in a mortar into a fine suspension in a salt solution. The suspension had a milky appearance, but particles could not be detected. The organisms were killed by heating in a water bath at 60 C. for thirty minutes. The sterility of the vaccine was confirmed by failure of large quantities of the heated organisms to grow on agar containing veal infusion and glycerin, on which living organisms grew readily.

The four following methods of administering the vaccine were studied and compared: injection of (1) living organisms subcutaneously, (2) living organisms intravenously, (3) heat-killed organisms subcutaneously and (4) heat-killed organisms intravenously.

A series of rabbits were also given injections of living BCG directly into the lung through the trachea by means of a long needle. The purpose of this manner of administering the BCG was to study the comparative effects of allergy and resistance in the pathogenesis of tuberculosis.

The four methods of giving the vaccine were suggested by results obtained from vaccinating rabbits with streptococci. In the experiments on vaccination with streptococci I¹² found that the intravenous method of administering the vaccine proved to be superior to the subcutaneous method in developing a higher degree of resistance, as demonstrated by the greater rate of destruction of organisms in the blood and liver and by a much higher agglutination titer. It was also shown that the intravenous method had an advantage over the subcutaneous in not producing a measurable amount of hypersensitiveness (allergy) to streptococci in the animals given injections and in desensitizing animals already hypersensitive. This greater degree of protection and the absence of allergy applied to both living and heat-killed streptococci when injected intravenously. The guinea-pigs were vaccinated subcutaneously with living organisms only. The amount given at each of the four weekly injections was 0.05 mg.

An attempt was made in the experiments reported in this paper to find the safest method of administering BCG vaccine which would result in the highest degree of resistance. The two chief considerations, therefore, were safety and resistance.

SAFETY

The factors of safety studied in vaccinating animals with BCG were (1) mechanical injuries, such as bacterial emboli in the brain or

12 Clawson, B. J. *J. Infect. Dis.* 53: 157, 1933.

other organs, (2) immediate or delayed toxic effects of the vaccine on normal or allergic animals, (3) whether or not allergy resulted and the significance of the allergy, and (4) development of lesions in the visceral organs (lungs, liver, spleen and kidneys) as a result of vaccination

Mechanical Injuries—These injuries, if present, would be most likely to be observed in the animals vaccinated intravenously with organisms, either living or heat-killed. The brain, lungs, liver, spleen and kidneys from many animals vaccinated intravenously or subcutaneously and killed for the purpose of dying of other causes were examined microscopically. In none of the organs of the animals were clumps of acid-fast organisms shown, and there was never any change in the tissues which gave evidence of embolism. At no time was there any clinical or morphologic evidence of ill effects from mechanical causes. From the standpoint of mechanical injury vaccination subcutaneously or intravenously with living or heat-killed B C G seemed to be safe.

Toxic Results—The possibility of toxic results was studied in normal animals and in animals which had been made allergic. In more than 250 normal animals vaccinated by one of the four methods and observed at periods of from a few weeks to as long as five months after the last injection of the vaccine there were no evidences of toxic effects, either immediate or delayed, other than those detected after injection of any other vaccine. The animals continued to be healthy and to grow and gain in weight.

Many animals were given a double dose of vaccine at each injection. Some were given many injections. Several were given intravenous injections of 1 mg. of living B C G at weekly intervals for as long as seventeen weeks. In none of the vaccinated animals were there at any time indications of toxemia due to vaccination.

The absence of toxic effects was observed only in normal animals. Different results were obtained in animals which had been made allergic and then given intravenous injections of B C G, especially in large doses. In the allergic animals marked toxic symptoms were noted in from twelve to twenty-four hours after an intravenous injection of a large dose of living or heat-killed B C G. In some of these animals death occurred (table 4).

Allergy—Another consideration for safety in vaccinating with B C G is whether an allergic state is developed in the course of vaccination. The term allergy as used in this paper is synonymous with hypersensitiveness to tuberculoprotein. This type of hypersensitiveness or allergy is exhibited as a delayed local reaction at the point of injection or as delayed general shock following an intravenous injection. The local reaction is indicated by redness, swelling and cellular infiltration fol-

lowing an intracutaneous injection of tuberculin and is called the Mantoux reaction. This type of hypersensitiveness was classified as "hypersensitiveness to infection" by Coca and Cooke¹³. Rich and Lewis¹⁴ referred to it as "an inflammatory-necrotizing type of hypersensitiveness to tuberculosis". Zinsser¹⁵ spoke of such hypersensitiveness in general as "bacterial allergy".

The two important things to know about allergy, if it is developed during a course of vaccination with BCG, is whether the allergic state may be dangerous and whether its presence is necessary for resistance. The significance of allergy in the pathogenesis of tuberculosis also has to be considered.

It is now generally believed that necrosis with its harmful results in tuberculous lesions is dependent on the allergic state. Long¹⁶ stated that the opinion is now growing more general that necrosis is largely the effect of the protein of the tubercle bacillus on a body made hypersensitive to the micro-organism by preexisting infection with the bacillus.

Rothschild, Friedenwald and Bernstein,¹⁷ from experiments in desensitizing tuberculous guinea-pigs with tuberculin, concluded that allergy was responsible for necrosis and mechanical spread in tuberculosis.

Birkhaug¹⁸ found that better protection was obtained from vaccinating guinea-pigs with BCG if the animals were not infected with the human strain until the allergy due to vaccination had decreased.

Evidently much harm may result from necrosis in a tuberculous organ. The necrosis can be a means of wide dissemination of the bacilli with spread of the infection. It would seem, therefore, that allergy should be looked on as a harmful factor in tuberculosis and should be avoided in vaccination against tuberculosis if resistance can be developed as well without it.

There is a dispute among immunologists at present as to whether allergy is necessarily a part of the state of resistance in tuberculosis as well as in other diseases. It is commonly stated that without allergy there is no resistance in tuberculosis.

Rich and his associates¹⁹ concluded that it has not yet been proved that an allergic state is a necessary part of the general process of

13 Coca, A. F., and Cooke, R. A. *J. Immunol.* **8** 163, 1928.

14 Rich, A. R., and Lewis, M. R. *Bull. Johns Hopkins Hosp.* **50** 115, 1932.

15 Zinsser, H. *Bull. New York Acad. Med.* **4** 351, 1928.

16 Long, E. R. *Am. Rev. Tuberc.* **22** 467, 1930.

17 Rothschild, H., Friedenwald, J. S., and Bernstein, C. *Bull. Johns Hopkins Hosp.* **54** 232, 1934.

18 Birkhaug, K. E. *Am. Rev. Tuberc.* **27** 6, 1933.

19 Rich, A. R., Chesney, A. M., and Turner, T. B. *Bull. Johns Hopkins Hosp.* **52** 179, 1933. Rich, A. R., and Brown, J. H. *Proc. Soc. Exper. Biol. & Med.* **27** 695, 1930. Rich, A. R., Jennings, F. B., and Downing, L. M. *Bull. Johns Hopkins Hosp.* **53** 172, 1933.

immunity (resistance), tuberculous or otherwise. They showed that resistance in syphilis may be present when the reaction to the luetin test (indicating the allergic state) is negative. They also demonstrated by injecting into normal animals the serum of immune animals which were both resistant and allergic to the pneumococcus that the resistance was transferred to the normal animals while the allergic factor was left behind. Their experiments in desensitizing animals immune and allergic to the pneumococcus or to *Bacillus avisepticus* and later in demonstrating marked resistance in these desensitized animals also seem to show that allergy is not necessary in the phenomenon of protection. Similar results were obtained by Rothschild, Friedenwald and Bernstein by desensitizing guinea-pigs allergic to tuberculosis.

Branch and Cuff²⁰ were able by injecting heat-killed tubercle bacilli intravenously or intramuscularly to develop resistance in guinea-pigs to the human tubercle bacillus without associated allergy.

In experiments in vaccination with streptococci I found that general resistance to streptococcic infection could exist without allergy. It was also concluded from these experiments that allergy as related to general resistance in streptococcic infection is a useless and at times a harmful phenomenon.

The opinions for and against the necessity of allergy in the immune state in tuberculosis are illustrated by the writings of Zinsser and Mueller,²¹ Petroff and Stewart²² and Seibert.²³

Zinsser's concept of the relation of allergy and resistance is that the allergic state and increased resistance are parallel, and perhaps causally related, phenomena and that the substance on which allergy depends may possess protective functions differing, and based on another mechanism, from those possessed by antibodies.

Petroff and Stewart stated that it is now generally accepted that the resistance of animals to superinfection depends chiefly on the degree of the allergic state and that the absence of allergy, as demonstrated by the reaction to the intracutaneous tuberculin test, spells susceptibility to infection. They found by experiments that guinea-pigs sensitized with killed tubercle bacilli and later inoculated with living tubercle bacilli outlived normal guinea-pigs infected in like manner which were used as controls. The vaccinated pigs were allergic.

Seibert, on the other hand, from experiments on sensitization with tuberculin protein concluded that a high degree of hypersensitiveness to tuberculin protein confers no immunity or increased resistance to

20 Branch, A., and Cuff, J. R. *J. Infect. Dis.* **47** 151, 1930.

21 Zinsser, H., and Mueller, J. H. *J. Exper. Med.* **41** 159, 1925.

22 Petroff, S. A., and Stewart, F. W. *J. Immunol.* **12** 97, 1926.

23 Seibert, F. B. *Proc. Soc. Exper. Biol. & Med.* **30** 1274, 1933.

subsequent tuberculous infections. On the contrary, it seems to hasten and extend the lesion and to be associated with much more extensive necrosis and caseation than are noted in nonsensitized animals. It is evident from these experiments that Seibert considered allergy in tuberculosis not only unnecessary to resistance but actually harmful.

Since it has been shown that allergy has a harmful aspect and that resistance may exist without allergy, it became important in the present experiments to study allergy as related to vaccination with B C G in an attempt to determine whether an efficient method of vaccination could be developed in which allergy was not produced. In the animals vaccinated by the four methods described allergy was studied in respect to (a) frequency and degree, (b) time of appearance after vaccination, (c) duration, (d) comparative duration of allergy and resistance, (e)

TABLE 1—*Frequency and Degree of Allergy in Rabbits Vaccinated by Four Methods*

No of Rabbits	Method	Allergy				Total Number Positive Reactions	Total Number Negative Reactions	Percentage Positive Reactions
		++++	+++	++	+			
35	Subcutaneous injection of living organisms	4	5	6	7	22	13	63
28	Intravenous injection of living organisms	2	1	4	9	16	12	57
26	Subcutaneous injection of killed organisms	0	0	2	8	10	16	38
42	Intravenous injection of killed organisms	0	0	0	0	0	42	0

effects of existing allergy in animals subsequently vaccinated, (f) relation to antibodies, (g) relation to resistance and (h) relation to lesions.

(a) The frequency and degree of allergy resulting from vaccination by the four methods are shown in table 1. Allergy was greatest both in frequency and in degree in the animals vaccinated subcutaneously or intravenously with living organisms. The degree was much less in the animals vaccinated subcutaneously with heat-killed B C G. No allergy occurred in the animals vaccinated intravenously with heat-killed organisms.

From the standpoint of the danger of producing allergy by the process of vaccination, the method of administering the vaccine intravenously as heat-killed organisms proved to be the one of choice. The degree of allergy in the group of animals vaccinated subcutaneously with heat-killed organisms was slight, and as shown later in the experiments, such allergy soon disappeared.

(b) The time of appearance of allergy carries with it a good deal of significance from the point of view of the effect of allergy on a

second infection. In most discussions of primary and secondary pulmonary tuberculosis it is assumed that the first type develops in the absence of allergy while the second is influenced in its progress by allergy. Rothschild, Friedenwald and Bernstein in experimental tuberculosis in guinea-pigs found that allergy appeared as soon as seven days after an infection with human tubercle bacilli.

In my experiments the animals were regularly tested for allergy three weeks after the last injection of the vaccine. If allergy had not appeared by this time it was found by many repeated tests that it did not appear. In 100 consecutive animals in which positive reactions to the Mantoux test were obtained allergy was present at the latest in three weeks after the last injection. It regularly appeared three weeks after a single subcutaneous injection of living organisms, and in one series

TABLE 2—*Duration of Allergy Resulting from Vaccination with BCG*

Number	Initial Degree of Allergy	Number of Months after Vaccination	Allergy, Degree
1	++++	3	++
2	++++	3	++
3	++++	3	0
4	+++	3	0
5	+++	3	0
6	+++	3	0
7	+++	1	0
8	++	2	0
9	++	1	0
10	++	1	0
11	+	1	0
12	+	1	0
13	+	1	0
14	+	1	0

tested allergy was present two weeks after the sensitizing injection. It may be said that allergy appears early in the development of lesions and that few if any tubercles observed are developed without the influence of allergy.

(c) The duration of allergy has a significant bearing from the standpoint of its effect on superinfection in the pathogenesis of tuberculosis. In table 2 is shown the duration of allergy in 14 vaccinated animals. It was noted that severe allergy, grades +++ and +++, due to vaccination with BCG disappeared in about three months. A less degree tended to disappear in a shorter time. It is probable that allergy associated with vaccination with BCG disappears more rapidly than the allergy resulting from an infection with virulent bacilli, for lesions due to BCG tend to heal more rapidly and, as has been shown, allergy seems to be dependent on lesions.

(d) The comparative duration of allergy and resistance has been a question of considerable dispute. It has commonly been assumed that without allergy there is no resistance. If this were true, according

to the aforementioned findings, resistance due to vaccination could last but about three months Willis,²⁴ in experimental tuberculosis in guinea-pigs, found the animals to be highly resistant to an inoculation of virulent bacilli after the allergy due to a preexisting infection with an organism of lower virulence had passed off

In table 3 are shown the results obtained for 8 rabbits in which the duration of allergy and resistance was measured and compared Three weeks after the last injection of the vaccine all 8 animals were allergic, from ++ to ++++ Just before the animals were given injections subcutaneously of 0.01 mg of virulent bovine bacilli the allergy had disappeared entirely in 4 rabbits and remained +++ in 4 The time between the vaccination and the injection of virulent organisms

TABLE 3—*Comparative Duration of Allergy and Resistance in Rabbits Vaccinated with BCG and Later Inoculated Subcutaneously with 0.01 Mg of Virulent Bovine Tubercle Bacilli (Ravenel)*

Number	Initial	Allergy, Injection of Virulent Bacilli		Resistance, Degree of Tuberculosis Three Months after Injection of Virulent Bacilli	
		Before*	Three Months After	Lungs	Kidneys
1	++	0	0	0	0
2	+++	0	0	0	0
3	+++	0	0	0	0
4	+++	0	0	0	0
5	+++	+++	0	0	0
6	+++	+++	0	0	0
7	+++	+++	0	0	0
8	++++	+++	0	0	0

* Inoculation was made from two to four months after vaccination

was from two to four months Three months after the injection of virulent bacilli, the time at which the animals were killed for examination, all gave negative reactions to the Mantoux test At this time none of the animals showed tuberculosis Nonvaccinated animals used as controls had extensive generalized tuberculosis From the observations in this experiment it was obvious that resistance can exist in the absence of allergy and may outlast allergy

(e) The effects of existing allergy on animals when subsequently vaccinated have to be considered carefully in the practice of vaccinating with BCG, especially if the intravenous method of vaccination is used Vaccination of persons against tuberculosis so far has been limited to nonallergic subjects It has been suggested from my experiments that vaccination of allergic persons might greatly increase the degree of resistance The question whether such vaccination could be done safely arose

In the discussion of toxic effects from vaccinating normal animals reference was made to the different results observed when animals already allergic were vaccinated intravenously, especially with large doses. The harmful results in such animals from the standpoint of toxic effects are shown in tables 4 and 5. Five rabbits were made allergic by giving each of them subcutaneously an injection of 1 mg of living BCG (table 4). Three weeks later these animals were tested for allergy. The reactions of the first 4 animals were + + +, + + +,

TABLE 4—*Degree of Allergy Obtained by Injecting Heat-Killed BCG Intravenously into Animals Previously Given Subcutaneous Injections of 1 Mg of Living BCG*

Animal	Amount of Killed BCG Injected, Mg	Result	Reaction to Mantoux Test
1	4	Death in 24 hours	+++
2	4	Death in 24 hours	++
3	4	Death in 24 hours	+++
4	4	Ill	+
5	4	No change	0
4*	3	Death in 24 hours	+
5	3	No change	0

* Experiments on rabbits 4 and 5 were repeated

TABLE 5—*Allergic Reactions Obtained by Four Weekly Injections Intravenously of Heat-Killed BCG (0.25, 0.5, 0.5 and 0.5 Mg) into Animals Previously Made Allergic by Injection of 4 Mg of Living BCG Subcutaneously*

Number	Reaction to Mantoux Test			Condition of Animal
	Three Weeks after Subcutaneous Injection	Two Weeks after Last Intravenous Injection	Four Weeks after Last Intravenous Injection	
1	++++	0	+++	Well
2	++++	0	+	Well
3	++++	0	+	Well
4	++++	0	0	Well
5	++++	0	0	Well

+ + and +, respectively. The fifth animal showed no allergy. Each rabbit then received an injection intravenously of 4 mg of heat-killed BCG. The first 3 animals collapsed after a few hours and died within twenty-four hours. The fourth became sick but survived. The fifth showed no ill effects from the injection. Mantoux tests showed that the fourth and fifth animals still gave reactions of + and —, respectively. Each animal was then given an intravenous injection of 3 mg of heat-killed BCG. The fourth animal died within twenty-four hours, and the fifth remained well.

Another experiment showed that with smaller doses allergic animals could be vaccinated intravenously without toxic results (table

5) In this experiment 5 rabbits were made allergic by a subcutaneous injection of 4 mg of living BCG. Three weeks after this subcutaneous injection the animals were tested for allergy and found to give + + + +, + + +, + + +, + + + and + + + reactions. They were then vaccinated intravenously with heat-killed BCG, first with 0.25 mg and on the three following occasions with 0.5 mg. Two weeks after the last injection of the vaccine all the animals were well and completely desensitized, as indicated by a negative reaction to the Mantoux test. Four weeks after the last injection of the vaccine the animals were still well, but allergy had returned in the first 3 rabbits in grades, respectively, of + + +, + and +. The results obtained in respect to toxic effects from vaccinating allergic animals with BCG showed that when the animals were given intravenous injections of large doses toxicity to the extent of producing death occurred. However, with small doses of heat-killed BCG administered intravenously allergic animals could be vaccinated without toxic effects. Such animals

TABLE 6—*Allergy and Humoral Antibodies in Animals Vaccinated with BCG*

Number	Allergy	Average Agglutination Titer	Average Complement Fixation Titer
10	++ to +++	1 375	1 250
15	0	1 200	1 230

for a time at least were desensitized. The possibility of injury to visceral organs in vaccination of allergic animals is discussed in connection with the relation of lesions and methods of vaccination.

The results described in the experiments on allergic animals demonstrate a danger which should be taken into consideration in vaccinating children with BCG, especially if they give a positive reaction to the Mantoux test.

(f) The relation of allergy to antibodies is significant in vaccination with BCG, for, as is shown later, an elevation of antibodies in the serum tends to be correlated with the development of resistance. If there is a similar correlation between allergy and antibodies it might be assumed that allergy could be used as an indicator of resistance or that the absence of allergy indicated the absence of resistance. Rich and Lewis and Aronson²⁵ showed by experiments with tissue cultures that allergy existed in the tissues independent of antibodies in the circulating blood.

In table 6 are shown the average agglutination and complement-fixation titers for groups of allergic and nonallergic animals. In 10 animals

allergic reactions in degrees of from ++ to +++ were produced. The average agglutination titer of these 10 animals was 1 375, and the average complement-fixation titer, 1 250. The second group of 15 animals had no allergy. The average agglutination titer of these 15 animals was 1 200, and the average complement-fixation titer, 1 230. The titers for the allergic animals were slightly higher than those for the nonallergic animals. This slight difference apparently was due not in any way to the allergy but to the fact that animals given injections of living organisms, especially intravenously, have higher antibody titers. The frequency with which such injections produce allergy is also high. The significant thing shown was that a relatively high antibody content could exist without associated allergy.

Another method of studying the relation of allergy to antibody content was by desensitizing allergic animals by means of intravenous injections of BCG (table 7). Five rabbits were made allergic by

TABLE 7—*Relation of Allergy and Humoral Antibodies in Desensitized Animals Which Had Been Allergic to BCG**

Number	Before Desensitization			After Desensitization		
	Reaction to Mantoux Test	Agglutination Titer	Complement Fixation Titer	Reaction to Mantoux Test	Agglutination Titer	Complement Fixation Titer
1	++	1 50	1 160	0	1 400	1 1,300
2	+++	1 50	1 160	0	1 400	1 1,500
3	+++	1 200	1 160	0	1 600	1 1,500
4	+++	1 200	1 160	0	1 800	1 1,500
5	+++	1 400	1 160	0	1 600	1 1,300

* Each of five control animals made allergic in a similar manner had a maximum complement fixation titer of 1 160, which dropped in three weeks to from 1 0 to 1 80.

injecting subcutaneously 4 mg of living BCG. Before desensitization of the animals by four weekly injections of heat-killed BCG the degree of allergy of 1 of these animals was ++ and that of 4, ++++. The agglutination titers before desensitization were 1 50 and 1 400. The complement-fixation titers were all 1 160. After desensitization all animals gave a negative reaction to the Mantoux test. The agglutination titers rose in the process of desensitization to from 1 400 to 1 800 and the complement-fixation titers to from 1 1,300 to 1 1,500. In 5 sensitized animals used as controls the maximum complement-fixation titer was 1 160, which dropped in three weeks to from 0 to 1 80. This experiment showed that desensitization to the extent of producing complete absence of allergy could be brought about by intravenous vaccination and that the content of antibodies was increased in the process of desensitization.

Experimental results given in table 8 show that a high degree of allergy can be present in the absence of antibodies. Ten animals were

made allergic by one large subcutaneous injection of living BCG. Three weeks after the sensitizing injection the animals were tested for allergy. The first 8 animals had allergy of grade + + +, the next 2, grade + +. The serums were tested for agglutinins at the same time. In none was there found a titer as high as 1:50.

What was found in regard to a proportionate or necessary relation between allergy and agglutinins and complement-fixing antibodies was also noted with respect to opsonins (table 9). It was shown in this

TABLE 8—*Allergy and Agglutinins in Animals Three Weeks After Subcutaneous Injection in One Area of 4 Mg of Living BCG*

Number	Reaction to Mantoux Test	Agglutination Titer
1	+++	0
2	+++	0
3	+++	0
4	+++	0
5	+++	0
6	+++	0
7	+++	0
8	+++	0
9	++	0
10	++	0

TABLE 9—*Average Number of Bacilli (BCG) Phagocytosed by One Hundred Normal Mononuclear Leukocytes in One Hour in the Presence of Normal or Immune Serums (Dilution 1:75), Agglutination Titers of the Serums*

Number of Animals	Kind of Serum*	Number of Bacteria per 100 Leukocytes	Agglutination Titers
4	Normal	25	0
10	KS	83	1:70
10	LS	90	1:60
10	Defatted I	122	1:240
10	KI	130	1:265

* KS indicates serum immunized with heat-killed organisms injected subcutaneously, LS, that with living organisms injected subcutaneously, I, that with living organisms injected intravenously, and KI, that with heat-killed organisms injected intravenously.

experiment that greater phagocytosis took place with the serums of the animals which received intravenous injections of defatted or heat-killed BCG and that in none of these animals was allergy ever found.

From the observations in the four experiments just described it was concluded that no definite proportionate or necessary relation exists between the presence of bacterial allergy and the concentration of antibodies, such as agglutinins, complement-fixing antibodies and opsonins. From other experiments the same thing can be said in regard to lysins.

(g) The relation of allergy to resistance has been discussed in general in connection with the literature on allergy. The absence of correlation between the presence of allergy and antibodies, already mentioned,

suggested that allergy was not necessary to resistance. One necessarily should be certain that resistance can exist without allergy before attempting to vaccinate so as to get rid of the harmful effects of allergy.

Observations were made in the present experiments which gave information on this point. In table 10 the relation of allergy and resistance is shown. Forty vaccinated animals were selected, all of

TABLE 10—*Allergy and Resistance in Animals Vaccinated with BCG and Later Inoculated Subcutaneously with 0.01 Mg of Virulent Bovine Bacilli (Ravencel)*

Number	Allergy, Degree		Resistance as Indicated by Degree of Tuberculosis in	
	Initial	When Inoculated	Lungs	Kidneys
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	+	0
11	0	0	++	0
12	0	0	++	0
13	0	0	++	0
14	0	0	++	0
15	0	0	++	0
16	+	0	0	0
17	+	0	0	0
18	++	0	0	0
19	++	0	+	0
20	+++	0	0	0
21	+++	0	0	0
22	+++	0	0	0
23	+++	0	+	0
24	+	+	0	0
25	+	+	0	0
26	+	+	0	0
27	++	++	0	0
28	++	++	0	0
29	++	++	0	0
30	++	++	0	0
31	+++	+++	0	0
32	+++	+++	0	0
33	+++	+++	0	0
34	+++	+++	0	0
35	+++	+++	0	0
36	+++	+++	0	0
37	+++	+++	0	0
38	+++	+++	0	0
39	+++	+++	0	0
40	+++	+++	0	0

which were later inoculated subcutaneously with 0.01 mg of virulent bovine tubercle bacilli. All showed either complete protection or only a slight amount of tuberculosis in the lungs, + on a basis of + + + +, ninety days after the injection of virulent bacilli. The degree of allergy was determined three weeks after the injection of the vaccine and again at the time of the injection of virulent organisms. Fifteen of the protected animals showed no allergy at any time, and 23 of the 40 animals were not allergic at the time of the injection of virulent

bacilli Resistance existed equally well in the absence of allergy and in its presence No obvious interdependence of allergy and resistance was noted The findings in the experiments justified the attempt to develop resistance by a method of vaccination which produced adequate resistance without the harmful factor of allergy

Lesions Due to Vaccination—Another factor of safety to be considered in vaccinating with BCG is whether lesions are produced in the visceral organs during the course of vaccination Many animals were vaccinated by the four methods already noted In table 11 are recorded the observations in respect to lesions produced in internal organs of normal animals by each method Microscopic examination revealed no lesions in the lungs, liver, spleen and kidneys after vaccination in any of the animals except in the group vaccinated intravenously

TABLE 11—*Frequency and Degree of Lesions in Visceral Organs of Animals Vaccinated by the Four Methods*

No of Animals	Methods	Lesions													
		Lungs				Liver				Spleen					
		++++	+++	++	+ 0	++++	+++	++	+ 0	++++	+++	++	+ 0		
10	Subcutaneously, living organisms	0	0	0	0 10	0	0	0	0 10	0	0	0	0 10		
12	Intravenously, living organisms	1	3	1	4 3	0	4	0	0 8	0	4	0	0 8		
10	Subcutaneously, heat killed organisms	0	0	0	0 10	0	0	0	0 10	0	0	0	0 10		
10	Intravenously, heat killed organisms	0	0	0	0 10	0	0	0	0 10	0	0	0	0 10		

with living organisms In this group of 12 animals lesions of grades + to +++ occurred in the lungs of 9 Lesions of grade +++ were present in the liver of 4 animals Four of the 12 animals had lesions in the spleen, all of grade +++ It would appear, because of the frequency and degree of lesions resulting from this method, that it should be contraindicated as a means of vaccinating against tuberculosis The other three methods in normal animals appeared to be safe

Lesions of slight degree in some cases developed in the internal organs of allergic animals when vaccinated intravenously with heat-killed organisms in doses which did not cause lesions or toxic effects in normal animals The lesions obtained by vaccinating allergic animals with small doses of heat-killed BCG are indicated in table 12 Six rabbits were made allergic by subcutaneous injection in one area in each animal of 4 mg of living BCG Three weeks after this sensitizing injection the animals were tested for allergy and found to give reactions of +++, +++, +++, ++, ++ and ++,

respectively. Each animal was then vaccinated with four injections of heat-killed B C G, the first dose being 0.25 mg and each of the three remaining doses 0.5 mg. Three weeks after the last injection of the vaccine the animals were killed, and the organs were examined microscopically for the presence of lesions. Microscopic lesions of grades from + to ++ were present in the lungs of all the animals and of grades from ++ to +++ in the liver of 3. No lesions were noted in the spleen or kidneys of any of the 6 animals. The greatest frequency and degree of involvement tended to be in the animals having the greatest degree of allergy. In 10 nonallergic animals vaccinated in a similar

TABLE 12—Results Obtained by Vaccination of Allergic Animals

A Allergic Reactions and Anatomic Changes Obtained by Four Weekly Injections Intravenously of Heat Killed BCG (0.25, 0.5, 0.5 and 0.5 Mg) into Animals Previously Made Allergic by Injection of 4 Mg of Living BCG Subcutaneously				
Number	Involvement of Lungs	Involvement of Liver	Involvement of Spleen	Reaction to Mantoux Test
1	++	+++	0	+++
2	++	++	0	+++
3	+	0	0	++
4	++	+++	0	++
5	+	0	0	++
6	+	0	0	++
B Allergy and Anatomic Changes Observed in Ten Animals Used as Controls Given Four Weekly Intravenous Injections of 1 Mg of BCG				
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0

manner, except that 1 mg of B C G was used in each injection, no lesions were detected in any of the organs. The experiments demonstrated an increased susceptibility to the development of lesions in allergic animals when given intravenous injections of heat-killed B C G. Repeated subcutaneous injections into many allergic animals always failed to produce lesions in the visceral organs. The increased susceptibility to lesions (though nonprogressive) noted in the allergic animals should be considered a dangerous factor in the vaccination of allergic persons. Further experiments are in progress to see whether by regulating the amount of vaccine given intravenously at each injection the development of lesions in the visceral organs can be prevented. The importance of the possibility of vaccinating allergic animals intravenously is obvious, for by this method of vaccination resistance in such animals is increased and desensitization is produced.

The following conclusions in respect to safety in the use of the four methods of vaccination with BCG can be stated. No mechanical injuries result from any of the methods. Immediate toxic effects do not occur. Delayed toxic effects occur only in allergic animals when vaccinated intravenously. Allergy occurred in greater degree and frequency in animals vaccinated with living organisms, subcutaneously or intravenously. Allergy was slight in animals vaccinated subcutaneously with heat-killed organisms. It did not occur at all in the animals given intravenous injections of heat-killed BCG. Allergy occurred early and soon passed off. It disappeared before resistance and bore no proportionate or causal relation to antibodies or resistance. In normal animals lesions (nonprogressive) in visceral organs were produced only by injecting living organisms intravenously, but in the allergic animals intravenous injections of heat-killed organisms produced a few lesions. Allergy was shown to have a harmful aspect and to be unnecessary for resistance. Except for the presence of allergy all the methods of vaccinating normal animals should be considered safe but the one in which living organisms are injected intravenously. The method in which heat-killed organisms were injected intravenously into normal animals was free from all the dangerous factors studied. Further experiments are needed concerning the safety of vaccinating allergic animals or persons, especially if the intravenous method of administering the vaccine is used.

RESISTANCE

Resistance is the chief factor to be considered in the use of BCG as a vaccine against tuberculosis, for unless a fair degree of increased resistance is developed vaccination, of course, is useless. Calmette and others have vaccinated a large number of children orally with BCG. Heimbeck's work demonstrated a degree of efficiency of BCG vaccine in reducing the number of cases of clinical tuberculosis developing among nurses exposed to open tuberculosis in the wards. Heimbeck vaccinated by injecting the living organisms subcutaneously. Park and his associates also vaccinated a large number of children subcutaneously with living BCG.

Birkhaug found that guinea-pigs vaccinated subcutaneously with living BCG lived longer than normal guinea-pigs inoculated with an equal amount of virulent human tubercle bacilli. Park and King²⁶ also reported that increased resistance was obtained by vaccinating animals with BCG against infections with virulent tubercle bacilli.

Resistance to tuberculous infection would necessarily have to be species-specific. Because of this fact I tried in my experiments to develop resistance to a virulent bovine strain (Ravenel) and to the

26 Park, W. H., and King, M. J. *Am J Pub Health* **19** 179, 1929

human strain (H 37) Rabbits were used in experiments with the bovine strain and guinea-pigs in those with the human strain The evidences of resistance were (1) elevation of the number of antibodies in the serum of the vaccinated animals and (2) protection of the animal against lethal inoculations of virulent bacilli The antibodies studied in relation to resistance were agglutinins, complement-fixing antibodies, opsonins and lysins

TABLE 13—Average Agglutination Titers in Rabbits Vaccinated by Four Methods

No. of Animals	Method	Agglutination Titers
22	Subcutaneous injection of living organisms	1 900
10	Intravenous injection of living organisms	1 700
38	Subcutaneous injection of killed organisms	1 100
53	Intravenous injection of killed organisms	1 300

TABLE 14—Degree of Tuberculosis in Normal and Vaccinated Rabbits Ninety Days After a Subcutaneous Inoculation of 0.01 Mg. of Virulent Bovine Tubercle Bacilli, Agglutination and Complement-Fixation Titers

No.	Normal Animals				Vaccinated Animals			
	Tuber- culosis in Lungs	Tuber- culosis in Kidneys	Agglu- tination Titer	Complement Fixation Titer	Tuber- culosis in Lungs	Tuber- culosis in Kidneys	Agglu- tination Titer	Complement Fixation Titer
1	+	0	0	0	0	0	1 50	1 160
2	++	0	0	0	0	0	1 50	1 160
3	+++	0	0	0	0	0	1 50	1 160
4	++++	+	0	0	0	0	1 50	1 160
5	++++	+	0	0	0	0	1 50	1 160
6	++++	+	0	0	0	0	1 200	1 324
7	++++	++++	0	0	0	0	1 400	1 324
8	++++	++++	0	0	0	0	1 800	1 648
9	++++	++++	0	0	0	0	1 800	1 648
10	++++	++++	0	0	0	0	1 3,200	1 648
11	++++	++++	0	0	0	0	1 400	1 1,295
12	++++	++++	0	0	0	0	1 600	1 1,295
13	++++	++++	0	0	0	0	1 800	1 2,500
14	++++	++++	0	0	+	0	1 50	1 80
15					+	0	1 50	1 160
16					+	0	1 100	1 160
17					—	0	1 200	1 160
18					+	0	1 800	1 160
19					++	+	<1 50	1 324
20					+++	0	1 200	1 648

The average agglutination titers for animals vaccinated by the four methods are recorded in table 13. The average maximum titer for the 22 animals vaccinated subcutaneously with living organisms was 1 300, for the 10 animals vaccinated intravenously with living organisms, 1 700, for the 38 animals vaccinated subcutaneously with heat-killed organisms, 1 100, and for the 53 animals vaccinated intravenously with heat-killed organisms, 1 300.

In table 14 is shown a comparison of the degree of tuberculosis in normal and in vaccinated rabbits ninety days after inoculating them subcutaneously with 0.01 mg. of the virulent bovine strain. The agglu-

mination and complement-fixation titers before the injection of virulent organisms are also given in each case. In none of the 14 normal rabbits were antibodies detected. Tuberculosis of degrees from + to + + + + was present in the lungs of all the rabbits. In only 2 of the animals was the degree less than + + +. Tuberculosis was present in the kidneys of all but 3 rabbits in grades of from + to + + + +.

The vaccinated animals in the main showed a decided elevation of antibody titer. Complete protection was noted in 13 of the 20 animals and, as compared with the normal animals used as controls, also in the 7 remaining rabbits. The lowest dilution tested for agglutinins was 1:50. All but 1 of the animals had a titer higher than this. The lowest dilution tested for complement-fixing antibodies was 1:30. All protected animals had a titer higher than this. As a rule, the content of antibodies in the serum was decidedly elevated in the vaccinated protected animals.

The average number of BCG bacilli phagocytosed by 100 normal mononuclear leukocytes in one hour at 37° C. in the presence of normal or immune serums is noted in table 9. The degree of phagocytosis is compared with the agglutination titer. The average number of bacteria phagocytosed was greater for the immune than for the normal animals. The animals vaccinated with defatted or heat-killed organisms showed the highest percentages of phagocytosis and also the highest agglutination titers. The degree of phagocytosis was correlated with the height of the agglutination titer.

The lytic action referred to in this paper is that indicated by the disappearance of organisms so that they are not shown either by acid-fast stains or by ordinary methods of staining. It seems to be necessary for the organisms to be phagocytosed before being lysed. The technic for determining the lytic power of a serum in the presence of mononuclear cells will be reported in another paper.

- The phagocytic power and lytic action of serums on BCG when mixed with mononuclear leukocytes are recorded in table 15. The lytic action greatly increased as the agglutination titer rose. The number of the nonlysed bacilli which were phagocytosed also greatly increased with the increase in the titer of the agglutinins.

The concentration of antibodies was increased by all the methods of vaccination. The findings in respect to phagocytosis and lysis suggested that the degree of destruction of organisms (lysis) was correlated with the degree of concentration of antibodies in the serums.

The final and surest proof of resistance was protection observed in animals against inoculation with lethal doses. In the rabbits the degree of resistance was measured by the amount of tuberculosis present in the lungs and kidneys from forty-five to ninety days after subcutaneous injection of 0.01 mg. of virulent bovine tubercle bacilli. These organs

were the usual sites of the first development of tuberculosis in rabbits. The amount of tuberculosis seen in the spleen, liver and kidneys of guinea-pigs was used as a standard to measure the protection in these

TABLE 15—*Phagocytic and Lytic Action of Normal Mononuclear Leukocytes on BCG When a Mixture of Equal Volumes of Leukocytes, BCG and Normal or Immune Serum (Dilution 1:75) is Incubated with Constant Mixing at 37 C for One Hour, Agglutination Titers of Serums*

Serums		Agglutination Titers	Average Percentage of Bacteria Remaining Visible after Incubation		
Number	Kind		Total	Inside Leukocytes	Outside Leukocytes
4	Normal	0	100	7	93
9	Immune	1:50 or less	71	19	81
4	Immune	1:100	45	25	75
6	Immune	1:200	40	19	81
2	Immune	1:400	20	31	69
4	Immune	1:800	15	86	14
2	Immune	1:1,600	8	80	20
2	Immune	1:3,200	8	92	8
3	Immune	1:6,400	5	95	5

TABLE 16—*Degree of Tuberculosis in Normal and Vaccinated Rabbits from Forty-Five to Ninety Days After Subcutaneous Inoculation with 0.01 Mg of Virulent Bovine Bacilli (Ravenel)*

No	Normal Rabbits		Rabbits Vaccinated with					
			Living BCG Subcutaneously		Killed BCG Subcutaneously		Killed BCG Intravenously	
	Lungs	Kidneys	Lungs	Kidneys	Lungs	Kidneys	Lungs	Kidneys
1	++	0	0	0	0	0	0	0
2	+++	0	0	0	0	0	0	0
3	+++	0	0	0	0	0	0	0
4	+++	+	0	0	0	0	0	0
5	+++	+	0	0	0	0	0	0
6	+++	+	0	0	0	0	0	0
7	+++	++	0	0	0	0	0	0
8	+++	+++	0	0	0	0	0	0
9	+++	+++	0	0	+	0	+	0
10	++++	+++	0	0	+	0	+	0
11	++++	+++	0	0	+	0	+	0
12	++++	+++	+	0	+	0	++	+
13	++++	++++	+	0	+	0	++	+
14	++++	++++	+	0	+	0	++	+
15	++++	++++			++	0	++	++
16	++++	++++			+++	0	+++	+
17					+++	+	+++	++
18					++++	++++	+++	+++
19							++++	+++
20							++++	+++

animals. It was noted that, as a rule, tuberculosis in guinea-pigs developed in these organs in the order of frequency named.

The degree of tuberculosis in normal rabbits and in rabbits vaccinated by three of the aforementioned methods from forty-five to ninety days after a subcutaneous inoculation of 0.01 mg of the bovine strain is shown in table 16. On account of the large amount of allergy and the

frequency and degree of lesions in animals vaccinated intravenously with living organisms this method was dropped early from subsequent experiments. A marked degree of resistance was noted in rabbits vaccinated by all three methods. It is doubtful from these experiments whether, from the standpoint of resistance, one method can be said to be superior to another.

Injection into the lung through the trachea of 1 mg. of living BCG was made in 10 rabbits (table 17). Later, these 10 and 4 normal animals were inoculated subcutaneously with 0.01 mg. of virulent bovine tubercle bacilli. The injections were made on the same day from the same suspension. All animals were killed forty-five days after the injection of virulent bacilli. The normal animals showed extensive tuberculosis.

TABLE 17—*Degree of Tuberculosis Forty-Five Days After a Subcutaneous Inoculation of 0.01 Mg. of Virulent Bovine Tubercle Bacilli (Ravenel) in Normal Rabbits and in Rabbits Which Had Previously Been Given Injections in the Lung of 1 Mg. of Living BCG*

Number	Normal Rabbits		Rabbits Receiving Injections of BCG in the Lung	
	Lungs	Kidneys	Lungs	Kidneys
1	+++	0	0	0
2	+++	++	0	0
3	++++	+++	0	0
4	++++	++++	0	0
5			0	0
6			0	0
7			0	0
8			0	0
9			+	0
10			+	0

The animals previously given injections of living BCG into the lung were all free from tuberculosis, except 2 which showed a + degree in the lung only. Marked resistance was noted in the animals previously vaccinated with BCG. In several of these animals a nodule or two were present in the lung at the point of injection of the BCG. Eighty per cent of such animals were allergic as a result of the injection. This experiment gave useful information in regard to the possible advantage of a healed Ghon tubercle in the lung.

The protection given to guinea-pigs against infection with the human strain (H 37) by vaccinating the animals with living BCG is indicated in the observations recorded in tables 18 and 19. In the first group (table 18) there were 18 normal and 15 vaccinated guinea-pigs. They died or were killed after inoculation with H 37 at periods ranging from thirty-two to ninety-six days. They were then examined for gross and microscopic evidence of tuberculosis. The degree of tuberculosis was estimated from the amount present in the spleen, liver and lungs. Tuberc-

TABLE 18—*Degree of Tuberculosis and Allergy (Due to Vaccination) in Normal and Vaccinated Guinea-Pigs from Thirty-Two to Ninety-Six Days After a Subcutaneous Inoculation of 0.05 Mg of H 37*

Number	Normal Animals		Vaccinated Animals	
	Tuberculosis	Allergy	Tuberculosis	Allergy
1	++++	0	0	++++
2	++++	0	0	++
3	++++	0	0	++
4	++++	0	0	++
5	++++	0	0	++
6	++++	0	0	++
7	++++	0	0	++
8	+++	0	0	++
9	+++	0	0	++
10	+++	0	0	++
11	+++	0	0	+++
12	+++	0	0	++
13	+++	0	+++	++
14	+++	0	++++	++
15	+++	0	++++	+++
16	+++	0		
17	+++	0		
18	+	0		

TABLE 19—*Degree of Tuberculosis in the Organs of Normal and Vaccinated Guinea-Pigs After a Subcutaneous Inoculation of 0.05 Mg of H 37*

Number of Days after Inoculation	Normal Animals				Vaccinated Animals			
	Number	Spleen	Liver	Lungs	Number	Spleen	Liver	Lungs
27	1	++	0	0	2	0	0	0
						0	0	0
28 29	2	+	0	0	2	0	0	0
		+++	++++	0		0	0	0
30	2	+	0	0	2	0	0	0
		+++	+++	0		0	0	0
33 36	3	+++	+++	0	1	0	0	0
		++++	++++	0				
		++++	++	+++				
37	4	+++	0	0	1	+	0	0
		+++	+++	0				
		++++	0	+++				
		++++	+++	+++				
38 39	1	++++	++++	++	3	0	0	0
						0	0	0
						0	0	0
40	1	++++	++++	+++	2	0	0	0
						0	0	0
42 45	1	++++	++++	+++	2	0	0	0
						+	0	0
46 50	2	+++	+++	+	1	+++	0	0
		++++	++++	+++				
58	1	++++	++++	+++	1	0	0	0
60	11	0	0	+	12	0	0	0
		+	+	0		0	0	0
		0	+	++		0	0	0
		0	+	+++		0	0	0
		+++	0	0		0	0	0
		+++	++++	+++		0	0	0
		+++	++++	++++		0	0	0
		++++	++++	+		+++	0	0
		++++	++++	+		+	0	+
		++++	++++	++++		+++	0	0
		++++	++++	++++		++++	0	+
						++++	++++	++++

culosis was observed in all the normal guinea-pigs, grade + + + + was noted in 7, grade + + + in 10 and grade + in 1. Allergy was not present before the injection of virulent organisms in any of the guinea-pigs. Of the 15 vaccinated animals tuberculosis was absent in 12. It was present in the 3 remaining animals in grades from + + + to + + + +. Allergy resulting from the vaccination was present in grades from + + to + + + at the time of injection with H 37.

In the second group (table 19) 29 normal and 29 vaccinated guinea-pigs were inoculated subcutaneously with 0.05 mg. of H 37 in an area about half-way between the axillary and the inguinal region. The amount of tuberculosis in the spleen, liver and lungs of the animals of each group is shown for the various periods at which the animals died or were killed. Twenty-seven days is the shortest time in which tuberculosis developed in the nonvaccinated animals.

When animals from the normal or the vaccinated group died a corresponding number from the other groups were killed. Sixty days after the inoculation with H 37 all the animals still living were killed. All animals were examined grossly and microscopically for the presence and degree of tuberculosis. These vaccinated guinea-pigs showed a marked degree of resistance to the human tubercle bacilli.

COMMENT

A series of experiments is described concerned with immune reactions in experimental tuberculosis. The purpose of the study was to determine an experimental basis for the safest and most efficient method of using the Calmette-Guérin bacillus (BCG) in developing resistance to bovine and human tuberculosis. Another consideration in the experiments was the relation of the reactions included in immunity to the pathogenesis of tuberculosis.

In conformity with the course pursued in previous experiments on vaccination with streptococci, the BCG vaccine was administered as living organisms subcutaneously and intravenously and as heat-killed organisms in the same ways. The vaccine so administered was studied in respect, first, to safety and, second, to resistance. No mechanical injuries, such as bacterial emboli or any other kind of tissue injury, were at any time observed from vaccinating many animals by each of the four methods.

No toxic results, either immediate or delayed, were noted in the normal animals after vaccination. With large doses given intravenously to animals already allergic, extreme collapse and death within twenty-four hours took place, but ordinary doses were not accompanied by toxic effects in the allergic animals. Subcutaneous injections of the vaccine into allergic animals had no ill effects. In allergic animals vaccinated

with relatively small doses intravenously, small, nonprogressive tubercles frequently developed in the lungs, liver and spleen. It would seem, until further observations and experiments are made, that vaccination of persons should be limited to those not having a positive reaction to the cutaneous tuberculin test. However, it would be an advantage if it is found that allergic animals can safely be vaccinated intravenously, for in the process of vaccinating the allergic animals they are desensitized, at least for a time, and resistance is increased.

Allergy was studied as a factor in safety and also in respect to its influence in the pathogenesis of tuberculosis. The frequency and degree of allergy were greater after vaccination by methods in which the living organisms were injected, but even by these methods allergy did not develop in all animals. A relatively small amount of allergy was produced in the animals given subcutaneous injections of heat-killed organisms. No animals vaccinated intravenously with heat-killed organisms ever became allergic.

It was found that severe allergy following vaccination disappeared in about three months and less severe allergy in less time, usually in about one month. This disappearance of allergy was probably more rapid than in persons in whom allergy is so frequently due to arrested active lesions. But it seems probable that any allergy, especially of the smaller degrees, which develops in the course of vaccination with BCG should not be looked on as a serious handicap.

Allergy in association with vaccination and probably with the development of a tubercle appears much earlier than is generally thought. It has been found to appear in from one to three weeks. In these experiments if allergy had not appeared in three weeks after the last injection of the vaccine it was found that it would not occur. The fact that allergy appears so early has to be considered in the consideration of the reaction of primary and secondary tuberculosis. It would seem that tubercles which are not influenced by the allergic state are seldom seen.

The experiments showed that allergy definitely tended to disappear before resistance. This should be remembered if a positive result in a test for allergy is looked on as an indicator of immunity. It is also a fact to keep in mind in coming to a conclusion on the much disputed question of whether allergy is a necessary part of the immune (resistant) state.

The experiments seemed to show that there is no proportionate or necessary relation between allergy and the immune bodies agglutinins, complement-fixing antibodies, opsonins and lysins. In this respect, allergy in tuberculosis seems to differ from the Arthus phenomenon. A high antibody content could be developed in animals without allergy, on the other hand, a high degree of allergy could occur without measurable

antibodies. Allergic animals were desensitized so that they failed to give a positive Mantoux reaction, and in the process of desensitization the number of antibodies was greatly increased.

It was found that allergy never occurred in animals in which lesions could not be found. This supported the dictum of Krause²⁷ "No tubercles, no allergy." In vaccinating with BCG or with other preparations in order to avoid the development of allergy, some method should be used which will not cause lesions.

The significance of allergy in the immune state has caused a great deal of debate. These experiments supported the conclusions of those investigators who contend that allergy is not a necessary factor in resistance. This was shown by three methods: (1) by vaccinating animals so as not to produce any initial allergy, (2) by waiting for the allergy to disappear before inoculating an animal with a virulent strain of bacillus, and (3) by desensitizing allergic animals by intravenous injections of heat-killed BCG and then giving an injection of a virulent strain and observing the resistance in the absence of allergy. Allergy has dangerous aspects, such as allergic shock and increased susceptibility to the development of lesions, but when it is taken into consideration that allergy tends to occur in a relatively small percentage of cases and in small degrees or not at all by adequate methods of vaccination, it is doubtful whether such an amount of allergy should be looked on as a very serious condition.

Only one of the four methods of vaccination in normal animals resulted in producing small, nonprogressive tubercles in the lungs, liver and spleen. This was the method in which living organisms were injected intravenously. Living organisms injected subcutaneously, even into allergic animals, produced no lesions in the visceral organs. Small lesions were produced by injecting heat-killed organisms intravenously into animals already allergic.

In general it can be said, as far as safety in the process of vaccination with BCG is concerned, that all methods are safe except the one in which the living organisms are injected intravenously. Even by this method progressive tuberculosis does not occur, but too many nonprogressive tubercles develop in the lungs, liver and spleen after the administration of the vaccine to permit one to consider it a safe method. The observations in the experiments did not justify the injection of living BCG vaccine intravenously.

Evidence of resistance due to vaccination against the virulent bovine strain of the tubercle bacillus in rabbits and against the virulent human strain in guinea-pigs was shown in two ways: (1) by a correlated increase in the titers of agglutinins, complement-fixing antibodies,

27 Krause, A. K. *Tr Nat A Prev Tuberc* **17** 348, 1921

opsonins and lysins, and (2) by actually preventing or greatly retarding the development of tuberculosis in rabbits and in guinea-pigs after inoculations with virulent strains of tubercle bacilli

CONCLUSIONS

Vaccination of nonallergic animals by any of the four methods described is not followed by mechanical injuries or by immediate or delayed toxic results. With large doses of the vaccine administered intravenously to allergic animals toxic effects may follow, but with moderate doses no delayed toxicity is noted.

Allergy is greatest in frequency and degree in animals vaccinated subcutaneously with living B C G, next greatest in animals vaccinated intravenously with living B C G and least in animals vaccinated subcutaneously with heat-killed B C G, but it does not occur at all in animals vaccinated intravenously with heat-killed B C G.

Allergy appears at the latest in from two to three weeks. It tends to disappear in from one to three months, depending on the degree.

Allergy never develops in the absence of lesions. The susceptibility to the development of lesions is increased by existing allergy. Allergy disappears more rapidly than coexisting resistance. There appears to be no proportionate or necessary correlation between the presence of allergy and the existence or concentration of antibodies in the serum. Allergy bears no proportionate or necessary relation to resistance. Definite resistance may be obtained to infection with bovine or human strains by vaccinating with B C G. The degree of resistance tends to be correlated with concentration of the antibodies in the blood.

Resistance coexistent with allergy more than compensates for the harmful effects of allergy.

The experiments suggest the possibility of safe and efficient vaccination of persons with B C G against ordinary degrees of infection with bovine or human strains of tubercle bacilli.

ACUTE ULCERATIVE ESOPHAGITIS

A PATHOLOGIC AND CLINICAL STUDY OF EIGHTY-TWO CASES OBSERVED AT NECROPSY

ELMER C BARTELS, M D

BOSTON

Whether certain changes in the esophagus which are found at post-mortem examination take place before or after death has been a subject of controversy since Hunter,¹ in 1786, reported his observations on self-digestion of the esophagus. Pringle, Stewart and Teacher² reviewed the whole subject in 1921. None of the many articles which have been written in the interim will be mentioned. Moutier,³ in 1921, reported 3 cases of acute postoperative esophagitis. Subsequently Henke and Lubarsch⁴ and Bell⁵ have contributed to the subject.

MATERIAL STUDIED

The material for this study consisted of pathologic specimens and clinical data gathered at the Mayo Clinic over a period of about seven years. Among 6,000 fresh and preserved esophageal specimens which were obtained at necropsy, 82 instances of the condition which is designated here as acute ulcerative esophagitis were found, an incidence of 0.013 per cent. Because of the direct information regarding these patients, it was possible to attempt correlation between the clinical and the pathologic observations.

PATHOLOGIC STUDY

In all of the cases, the esophagus was found to be dilated at necropsy, especially in the lower third, and to contain gastric contents. The flow between the stomach and the esophagus was uninterrupted,

From the Department of Pathology, the Mayo Foundation, Rochester, Minn.
Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine, June 1932.

1 Hunter, John. The Works of John Hunter, London, Longman, Rees, Orme, Brown, Green and Longman, 1835, vol. 1.

2 Pringle, J. H., Stewart, L. T., and Teacher, J. H. J. Path. & Bact. **24** 396, 1921.

3 Moutier, F. J. A. M. A. **76** 1536, 1921.

4 Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer 1926, vol. 6 pt. 1.

5 Bell, E. T. A Text-Book of Pathology, Philadelphia, Lea & Febiger, 1930, p. 459.

as the supposed cardiac sphincteric action was not present. Ulcerations were not found in any case in which the esophagus was firm with the normal, folding rugae present. In specimens which had been subjected to the stretching, dilating force of the contained fluid, these folding rugae were obliterated.

The wall of the esophagus usually was pliable and thinned, and in some cases the color of the fluid which it contained could easily be made out. The mediastinum was involved in only 3 cases. In 2 of these cases perforation had taken place into the pleura, and in the other case purulent mediastinitis apparently had followed extensive ulceration of the esophagus and was a contributing cause of death. The mediastinitis was partially localized around the lower third of the esophagus.

The changes which were seen when the esophagus was opened usually were limited to the entire circumference of the lower third or half of the viscus. In only 2 cases was almost the entire esophagus involved.

The type of lesions, from the gross standpoint varied greatly and may be described as follows: (1) phlegmonous and pseudomembranous ulcerations, (2) irregular superficial coalescing ulceration with hemorrhage or with bile staining, (3) linear or longitudinal ulceration with hemorrhage or black eschar, (4) simple erosions, petechiae-like lesions with congestion, and (5) perforating ulcerations, which varied in size and shape.

The extent of the ulcerative change varied somewhat. Occasionally the ulceration was moderate, but in most cases it was diffuse and widespread, leaving only a small amount of mucosa, in island-like formation. The residual mucosa appeared somewhat softened and could be rubbed off by pressure of the finger leaving the submucosa and muscularis.

The gross appearance usually predicated the type of lesion that would be seen microscopically, but this was not uniformly true, for the autolysis incident to postmortem changes altered the true picture and minimized the inflammatory nature of the lesion. Microscopically the ulcerations could be grouped into four main types: (1) pseudomembranous ulceration, (2) simple ulceration with slight or marked inflammatory change, (3) hemorrhagic ulceration, and (4) phlegmon of the entire wall, similar to that which accompanies perforation.

Pseudomembranous ulceration was characterized microscopically by more or less replacement of the normal squamous mucosal layer with one which consisted of cellular and fibrinous debris, which might be stained with bile. Beneath this layer there was found evidence of infiltration of the remaining submucosa by leukocytes, mostly polymorphonuclears. The degree of infiltration might be slight or so extensive that the entire submucosa and muscularis were involved. The

blood vessels, if present, usually were not engorged, and there was little, if any, hemorrhagic infiltration of the tissues. This type of ulceration rarely bled.

Simple ulceration was characterized by denuding the tissue of the squamous layer of epithelium, without replacement (fig 1A). The submucosa was infiltrated with an inflammatory cellular exudate. At times, this was mild, but it might be severe and involve all the layers down to the muscularis. Here and there, small masses of degenerating erythrocytes might be seen. In cases in which there was severe infiltration, there were masses of necrosis in the submucosa. The nuclei of the normal tissue stained poorly.

Hemorrhagic ulceration (fig 1B and C) represented the type that accounted for most of the bleeding, especially of the profuse type. The layer of squamous epithelium was lost. In the submucosa, the vessels were dilated and engorged, and erythrocytes infiltrated the tissues. Edema of the tissues usually was marked, and leukocytic infiltration varied from slight to moderate, but was never extensive. Occasionally, eosinophils were found to be numerous.

It was not difficult to understand the ease with which hemorrhage had been induced in these cases, when it was seen that the vessels were situated close under the squamous layer, especially if they were dilated, and that a slight inflammatory change would erode the walls of these vessels. Ulcerations might be responsible for the supposed spontaneous rupture of esophageal varices in cases of hepatic cirrhosis.

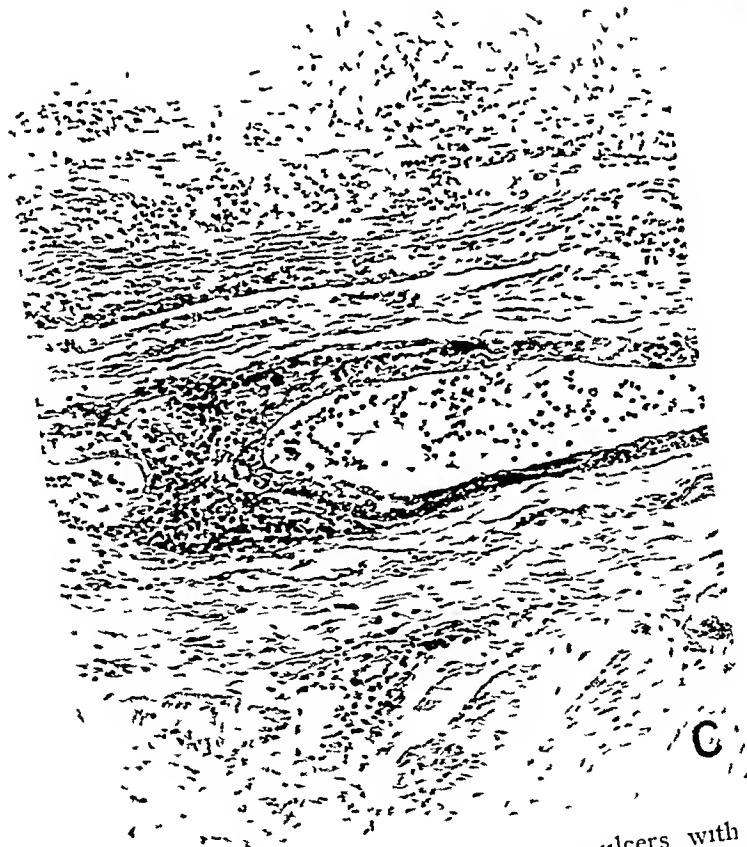
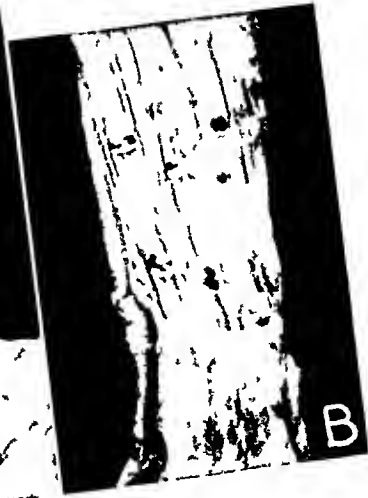
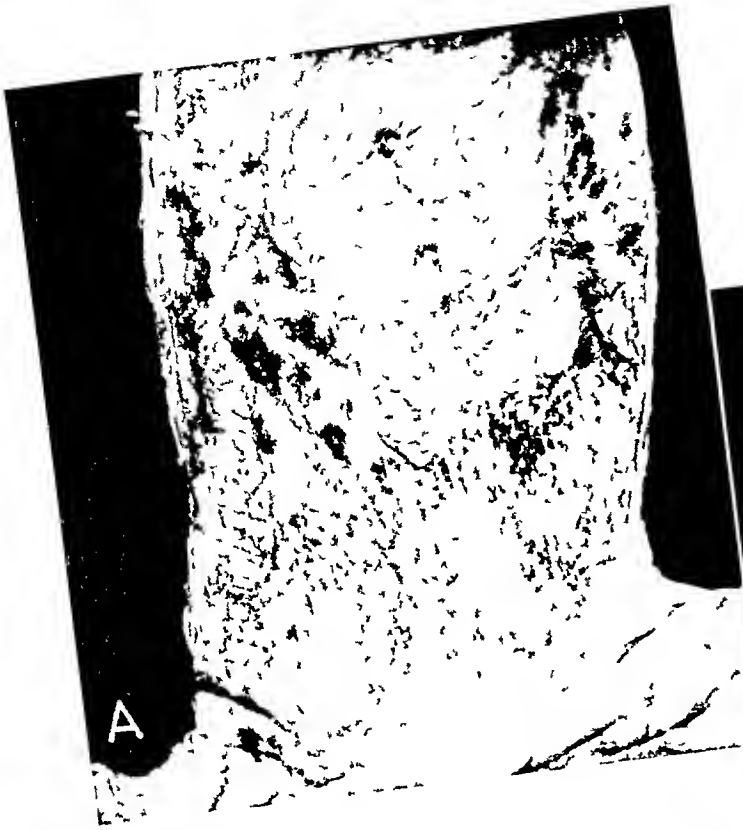
In phlegmonous ulcerations, which are diffuse types of ulceration, the normal constituents of the tissue were absent, and only leukocytic infiltration, in degenerating, necrotic, cellular debris, remained. The entire wall was thickly infiltrated by leukocytes for a considerable distance from the perforation, and there was gradual transition to the normal esophageal tissues.

The mucosa of the stomach was examined in a number of the cases in which there were severe changes in the esophagus, but inflammatory changes were not found. There was, however, slight digestion of the rugae, for they had lost some of their natural contour. This proves that there is some type of digestion which follows death, but this can be distinguished from antemortem conditions by the presence of inflammatory reaction in the latter.

CLINICAL STUDY

Pringle and Teacher⁶ were the first to recognize the clinical importance of this condition. Their cases were of postoperative hematemesis of esophageal origin. The cases included in the present report were

⁶ Pringle J. H., and Teacher J. H. *Brit J Surg* 6:523, 1919.



A, photomicrograph of multiple small hemorrhagic ulcers with many linear superficial ulcerations in the lower third of the esophagus, $\times 120$ *B*, numerous deep and superficial ulcerations of the lower third of the esophagus. The deep ulcerations are hemorrhagic *C*, hemorrhagic ulceration of the esophagus. The squamous layer is no longer present. There is a leukocytic infiltration throughout the submucosa, extending into the muscularis, and there is some edema of the tissues

grouped and studied from the following standpoints (1) primary cause of death, (2) age, (3) sex (4) whether or not vomiting occurred, and if so, whether or not blood was present in the vomitus (5) trauma from passage of a stomach tube, (6) symptoms referable to the esophagus, and (7) miscellaneous symptoms

Primary Cause of Death—Fifty-five deaths in this series followed operations. The remaining 25 patients who died were treated medically. The surgical cases were grouped as to procedures. In 13 cases, operation was performed on the colon, in 12, on the genito-urinary tract, in 8, on the stomach, in 13, on the gallbladder, bile ducts and pancreas, in 4, on the head, in 2, on the neck, including the thyroid gland, and in 10 on miscellaneous organs.

This grouping, because operations on the head are relatively infrequent in a surgical practice suggests that esophagitis is slightly more likely to follow such operations than it is to follow others. The literature tends to bear this out. However, this is only suggestive. The presence of esophagitis in association with lesions of the brain proves that the prevalent surgical complication of general peritonitis is not an essential factor in the etiology of esophagitis. However, it might be said that all the operations were rather difficult and extensive, and occasionally, the patients constituted poor surgical risks.

The length of time between operation and death did not seem to be of significance, for the esophagitis appeared from one to fifteen days after operation, and in twenty cases it appeared later than this. Its severity also bore no relation to the number of days that had elapsed since the operation. High fever was a common clinical manifestation.

In the cases in which medical treatment was employed, acute ulceration of the esophagus occurred as follows: in 13 cases of sepsis or infection, in 6 cases of heart disease, and in 1 case each of tuberculosis, diabetes, uremia, fracture of the ribs with hemorrhage, leukemia and carcinoma of the pancreas. All these conditions were debilitating, and the patients died after prolonged illness, except a patient who had coronary disease and died twenty-four hours after an infarction. There were no sudden deaths in this group.

That the condition should be present in 6 cases of chronic ulcerative colitis seems to be more than coincidental and might suggest the possibility of susceptibility to ulceration. On the other hand, most of the patients were severely debilitated and 5 of them had had operations on the colon.

In a few cases mercuriochrome had been administered intravenously because of sepsis and in addition to ulceration of the esophagus, the rather typical ulcerations of the colon were present. Ulceration of the esophagus however occurred in too small a number of cases in which

mercuriochrome was injected intravenously to warrant drawing any conclusion

Age—The age of the patients varied from 8 months to 78 years, and although it is difficult to judge its significance, because of numerous variables, it is not felt to be of any significance as an etiologic factor. The patients were distributed rather evenly in the adult periods. Three patients were between 8 months and 9 years of age, 2 were between 10 and 19 years, 10 were between 20 and 29 years, 12 were between 30 and 39 years, 13 were between 40 and 49 years, 18 were between 50 and 59 years, 15 were between 60 and 69 years, 8 were between 70 and 79 years of age, respectively, and 1 patient was aged 82 years.

Sex—Twenty-four of the patients were females and 58 were males. However, males outnumbered females in the general registration at the clinic, and consequently more males than females were subjected to operation. The number of deaths from surgical procedures which involved the genito-urinary tract was also high and chiefly affected males. I feel that the factor of sex is of no significance.

Vomiting—Gross vomiting occurred in 59 cases, and in the remaining 23 cases, little, if any, vomiting was observed. The amount of vomitus was extremely variable. In 18 cases, it was considerable, and in the remainder it was only slight or moderate. Vomiting had been present from one to fourteen days in most cases, and in only 4 had it persisted as long as six weeks, and then only at intervals.

Blood was prominent in the vomitus in 15 of the 59 cases in which vomiting occurred. It was difficult or impossible to ascertain the amount of blood vomited, for considerable gastric content, which could not easily be separated, was associated with the vomitus. Amounts of gastric content which contained blood varied from 50 to 2,000 cc. The vomitus was usually described as having had the appearance of coffee grounds, but in 1 case it was bright red. In none of the cases in which blood was present was there a lesion of the upper part of the gastro-intestinal tract or lungs that could account for it. Therefore, the natural deduction is that it unquestionably came from the lesions in the esophagus, which could well be responsible. For this reason, vomiting of blood indicates ulcerations of the esophagus in the known absence of gastro-intestinal or pulmonary lesions.

The vomiting of blood, although definite, usually did not seem to cause much concern, probably because of the serious condition of the patient at the time, when nothing could be done if the source had been ascertained, and also because the bleeding did not seem to be a serious complication, except in rare cases. Vomiting of blood caused faulty conclusions once. The patient had painless jaundice for five weeks following an attack of so-called ptomaine poisoning. Vomiting had

been present at intervals for four and a half weeks. In the hospital, the drainage from the duodenum did not reveal bile or blood, and a diagnosis of carcinoma of the head of the pancreas was made. The vomitus soon turned from dark to black (blood), and the possibility of the malignant lesion eroding into the stomach or duodenum with consequent bleeding was considered. At necropsy, it was found that this condition had not occurred. The bleeding unquestionably had proceeded from the diffuse ulcerations of the esophagus, which microscopically gave evidence of extensive inflammatory changes at the base of the acute, discrete, superficial, bile-stained ulcerations. This case suggests the clinical significance of the lesion.

In another case, esophageal bleeding following ulcerations into esophageal varices was the contributory cause of death. The patient was a woman, aged 68 years, who entered the clinic with a history of having had slight icterus for four months following an attack of influenza and colic of the gallbladder. Cholecystectomy and choledochostomy were performed for empyema of the gallbladder and stones in the gallbladder and ducts with impaction. The patient made an uneventful recovery until the fourth day, when she passed six tarry stools and became dyspneic. The pulse became rapid, and she failed rapidly. At necropsy, the stomach and intestines were found to be filled with blood. No source of the bleeding could be found other than acute ulcerative esophagitis, which had caused erosion of esophageal varices. Microscopic study proved the premise, for typical ulcerations were found, with erosions into the dilated vessels of the submucosa. This case also suggests the range of clinical importance of this pathologic lesion.

At necropsy, blood was found in the stomach in 7 cases. This varied in quantity from 200 cc. to so much that the stomach was described as full of blood. Here again, with the exception of a case in which an apparently clean gastro-enterostomy had been performed for a duodenal ulcer which did not bleed, no source for the blood except the esophagus could be found. The blood was described as having had the appearance of coffee grounds, or as having occurred in clots, therefore there was no doubt as to its presence.

Trauma—Trauma from intubation has been thought to be a factor in the production of these lesions, but close investigation of the case histories revealed that this could have occurred in only 30 cases, or about a third of the fatal cases. In the cases in which a tube had been passed into the esophagus, the lesions apparently were not different from those in which this had not been done. At first the linear ulcerations seemed to be the result of the intubation, but this again was not proved in this study. This warrants the conclusion that passage of a

tube is not an etiologic factor and may only incidentally be an aggravating one. One patient suffered considerable pain when the tube was passed.

Miscellaneous Symptoms—Miscellaneous symptoms referable to the esophagus were present in 11 cases, and were grouped as follows: dysphagia, which occurred in 7 cases, and high epigastric pain or burning, which occurred in 4 cases. There were 5 cases in which there was severe to intractable hiccup, without peritonitis, pneumonia or other cause of phrenic irritation. Because of the fact that hiccup is occasionally an early, or even the first, symptom of carcinoma of the esophagus or of cardiospasm, it is probable that hiccup represents a symptom of ulceration of the esophagus. Therefore, I wish to include it and bring the number of cases with miscellaneous symptoms up to 16.

Dysphagia, although usually severe, can be qualified by the following instances: 1. One patient refused food because of the severe pain associated with swallowing. 2. "Burning all the way down the esophagus" was so severe in another patient's case that a laryngologist was called, and esophagoscopy was suggested. 3. A presumptive diagnosis of carcinoma of the esophagus was made once because of marked dysphagia, which was present at the time of the patient's admission to the hospital, and because of vomiting that had occurred previously. The patient died of uremia, which was caused by polycystic kidneys. 4. Lower substernal pain was so severe in 1 case that the question of disease of the gallbladder was considered. 5. Passage of a tube caused pain in 1 case in which dysphagia already was a symptom. 6. In 2 cases, burning, which was situated high in the epigastrium, was present without any apparent reason. Dysphagia or burning in the esophagus was present in only 11 of the 82 cases, this seems a very small proportion; on the other hand, when it is realized that these symptoms were complained of without the patients being questioned, it can be assumed that it was present in many more than 11 cases. There can be no doubt that ulceration, because of the symptoms mentioned, occurs without causing death and consequently does not represent merely a terminal affair, for this reason, the condition is of clinical significance.

It has been suggested that anesthesia has something to do with esophagitis. Moutier reported a case in which the patient had swallowed the anesthetic. However, esophagitis occurred in cases in which operation was not performed, and in the surgical cases the types of anesthesia were extremely variable, a large number of patients received local and spinal anesthesia. This suggests that anesthesia is not a predominant etiologic factor. In cases in which general anesthesia was used, the extent and type of ulceration were in no way different from those in other cases.

Perforation occurred in 2 cases, and in these there were no symptoms to indicate that the accident had occurred, and dysphagia was not present. Unquestionably, the perforation took place just before death, for there was no inflammatory reaction in the pleural cavity or mediastinal tissues. That the perforation was aided by the ulceration is proved by the fact that microscopic study of the edge of the perforation revealed inflammatory changes, which extended through the entire esophageal wall.

Mediastinitis, which was present in 1 case, produced dyspnea and pain in the upper part of the abdomen. The mediastinitis seemed to be secondary to the ulceration, for the entire wall of the esophagus was uniformly infiltrated beneath the ulceration, and the same process seemed to extend into the mediastinum. Because the patient had empyema, it might be inferred that this was the primary source of the mediastinitis, but there was no evidence of a communication between the pleura and the mediastinum. Also, the condition was really periesophageal, and involved only the lower third of the mediastinum, which was in the region in which the esophageal changes occurred.

COMMENT

As a result of the pathologic study, there seems to be no doubt that the term "acute ulcerative esophagitis" describes the condition which was found in these cases better than does the term "esophagomalacia," or "intravital softening." In all the cases there were, without a doubt, inflammatory changes. It seems imperative that the action of the gastric juice be accepted as contributing to the lesion, because gastric juice was present uniformly in the lower third of the esophagus. Vomiting and even nausea without vomiting, with relaxation of the cardiac spasm, seem extremely important as factors that permit gastric juice to come in contact with the esophagus.

It seems necessary that the patient should be debilitated, but not necessarily dying, before changes can occur in the esophagus. Debilitating and terminal states imply slowing of the circulation of the lower part of the esophagus, which normally is poor, and, therefore, loss of the normal resistance of esophageal tissue to trauma, and of its ability to regenerate. Thrombosis of small vessels has been suggested as a factor, but that it occurs has not been proved by microscopic study. Normally, as is known from experimental studies, the esophagus has good ability to repair any area that is traumatized, and the only conclusion which can be drawn is that in the cases which comprise this study the body was unable to carry on its work efficiently.

From a clinical standpoint, esophagitis should be recognized as a condition that is able to cause symptoms and to produce signs, as has

been brought out by close investigation of patients while they were under observation in the hospital. There is reason to believe that this condition is on the increase, for several years ago Verbruggen did not find a significant number of cases. Of late, it is being found more often than it was found formerly, and there is no possibility that this frequency should have come about merely because it was not looked for as diligently in former years as it is at present. I have no explanation to offer for the increasing prevalence.

SUMMARY

The term "acute ulcerative esophagitis" is more appropriate and is suggested instead of the term "esophagomalacia," or "intravital softening," which was used previously.

The condition produces ulcerative changes in the lower part of the esophagus, these changes, both gross and microscopic, are described.

Acute ulcerative esophagitis is of clinical significance because of its symptoms and signs. It also seems to be more prevalent than it was formerly.

Peptic ulcer (so-called) of the esophagus was not encountered. Scars which would suggest its previous existence were not seen.

LYMPHOMATOSIS IN RELATION TO FOWL PARALYSIS

J FURTH, M D

WITH THE ASSISTANCE OF CHARLES BREFDIS

NEW YORK

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The etiology of lymphomatosis, the most common neoplasm of the domestic fowl has been investigated by many workers familiar with

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These investigations have been supported by a Fund for the Study of Leukemia

both filtrable viruses and neoplasms, but without success. When Ellermann and Bang¹ observed that erythroleukosis and myeloblastic leukemia are caused by a filtrable virus they discovered a new filtrable virus as well as new types of leukosis in the domestic fowl. They believed that lymphoid leukosis was also caused by their virus, but Anderson and Bang² failed to transmit lymphoid leukosis, and the few instances of this disease occurring among the passages of the virus of Ellermann were probably spontaneous.³

Recent experiments indicate that lymphoid leukosis may be caused by several viruses.⁴ The first virus (that of strain 2) which was shown to produce a lymphomatous neoplasm produced also endothelioma and myelocytomatosis. Lymphomatosis produced by this virus is associated with infiltration of nerves but with no clinical manifestations of paralysis.^{4a} In subsequent experiments we have transmitted lymphomatosis associated with extensive infiltration of nerves and with clinical manifestations of fowl paralysis, but this disease has seldom been associated with leukemia, and never with endothelioma or myelocytomatosis (strains 5 and 6^{4b}). Successful transmission of this variety of lymphomatosis has already been reported by Pappenheimer, Dunn and Cone,⁵ who have named it "neurolymphomatosis." Evidence will be presented here that these two types of lymphomatosis (strains 2 and 5) differ both etiologically and anatomically.

I LYMPHOMATOSIS PRODUCED BY STRAIN 2

BLOOD CHANGES

Most lymphocytes that circulate in the blood of normal chickens are small and represent approximately 61 per cent of all leukocytes. Their cytoplasm is pale, and in some cells in preparations stained with a combination of Wright and Giemsa solutions, it contains minute azurophil granules. An occasional small lymphocyte with basophil cytoplasm may

1 (a) Ellermann, V., and Bang, O. *Centralbl f Bakt* **46** 1, 1908. (b) Ellermann, V. *The Leucosis of Fowls and Leucemia Problems*, London, Gyldendal, 1921.

2 Anderson, C. W., and Bang, O. *Festskrift til Bernhard Bang*, Copenhagen, 1928, p. 355.

3 (a) Furth, J. *Proc Soc Exper Biol & Med* **27** 155, 1929, *J Exper Med* **53** 269, 1931. (b) Stubbs, E. L., and Furth, J. *ibid* **53** 269, 1931. (c) Engelbreth-Holm, J. *Ztschr f Immunitätsforsch u exper Therap* **75** 425, 1932. (d) Engelbreth-Holm, J., and Rothe Meyer, A. *Acta path et microbiol Scandinav* **9** 293, 1932.

4 (a) Furth, J. *J Exper Med* **58** 253, 1933, (b) *Proc Soc Exper Biol & Med* **31** 921, 1934.

5 Pappenheimer, A. M., Dunn, L. C., and Cone, V. *J Exper Med* **49** 63, 1929.

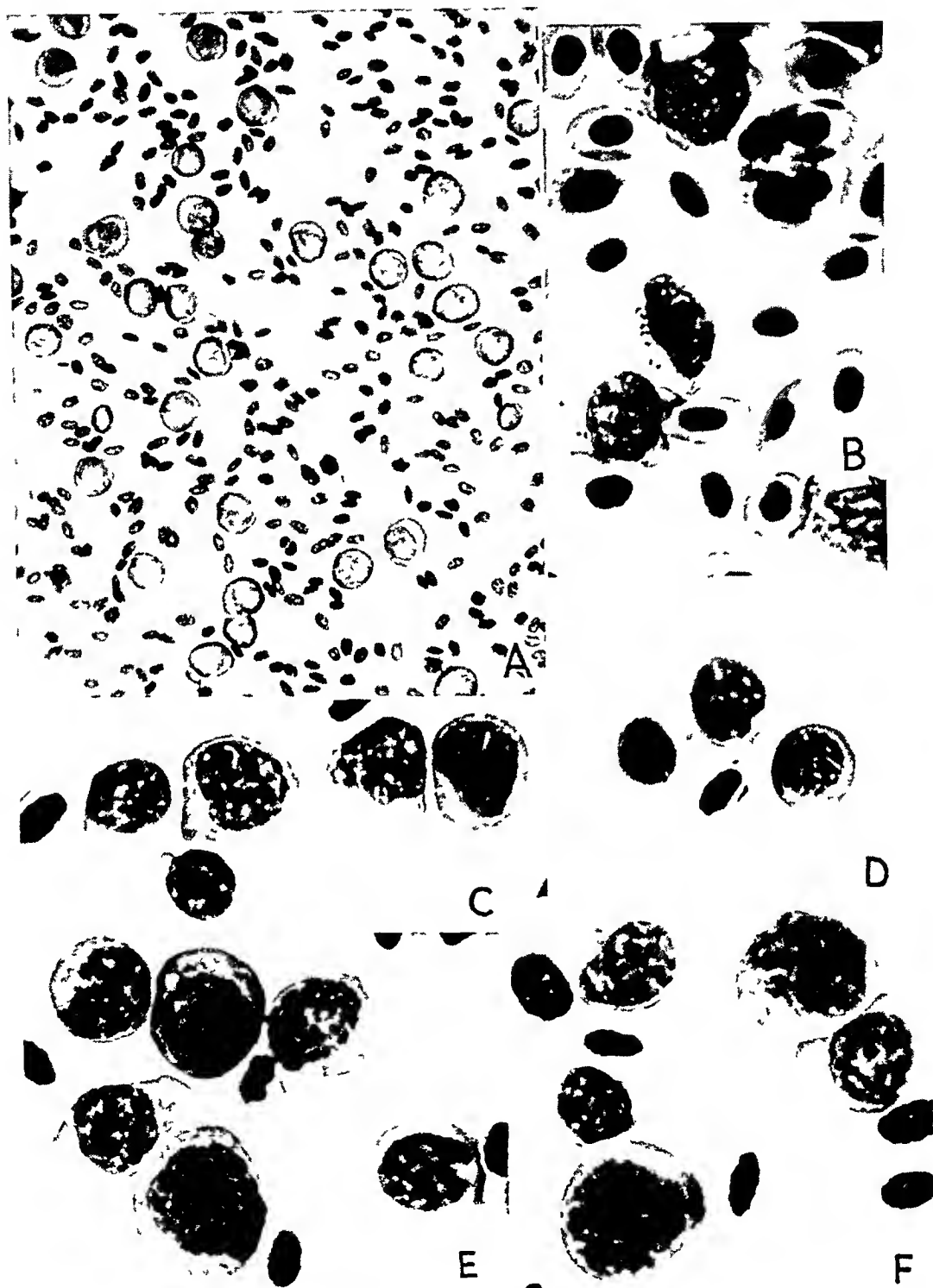


Fig 1—Blood cells in lymphatic leukemia produced by strain 2. The lymphocytes shown in *B* have minute azure granules. Transitional forms between large and small lymphocytes are seen in *C*. *D* shows a lymphocyte, an erythroblast and a thrombocyte. All blood smears were stained with Wright and Giemsa solutions. The magnifications are approximately *A*, $\times 400$, *B-F*, $\times 1000$.

be seen in the blood of normal chickens, but basophil lymphocytes of medium and large size are absent. Lymphomatosis of strain 2 is characterized by the presence in the blood of numerous basophil lymphocytes of medium and large size (fig 1 *A, B, C, E* and *F*). The cytoplasm of many of these cells contains vacuoles in some of which there are minute azurophil granules (fig 1 *B*). There are continuous transitional forms between the large lymphocytes of strain 2 and normal small lymphocytes (fig 1 *C*). Erythroblasts can be readily distinguished from the lymphocytes of strain 2, as shown in figure 1 *D* moreover with anemia, notably after intramuscular inoculations with the large lymphocytes. Severe anemia almost invariably accompanies lymphatic leukemia produced by this strain is occasionally unassociated early and extensive lymphomatous infiltration of the marrow. In most chickens with lymphomatosis of strain 2 the total number of leukocytes was slightly or moderately increased, in several chickens it exceeded 100,000 per cubic millimeter. The highest leukocyte count found was 1,200,000. Differential counts are shown in table 1.

ANATOMIC CHANGES

The tumors produced by intramuscular transmission of strain 2 are gray, somewhat yellowish, ill-defined neoplasm diffusely infiltrating adjacent muscle tissues. Most birds died with lymphomatosis of the internal organs from four to eight weeks after inoculation, when the tumors in the breast muscle measured from 1 to 4 cm across. The tumors were of meaty consistency and were usually free from hemorrhage and necrosis. They were formed by large lymphocytes (fig 2 *A* and *B*) which very readily invaded the blood stream. These cells were conspicuous in blood smears as early as two or three weeks after inoculation.

The liver, spleen and bone marrow were almost invariably the sites of extensive lymphomatous infiltrations, the thymus, kidneys, heart muscle, lungs, ovaries, nerves and ganglions were often infiltrated.

The liver was enlarged up to four times its normal size in most instances, gray mottling (fig 3 *A*) caused by perivascular lymphomatous infiltration (fig 4 *A*) distinguished lymphomatosis of this organ from erythroleukosis of strain 1, which is associated with a diffuse intravascular accumulation of erythroblasts or myeloblasts (leukostasis). Leukosis of strain 2 is characterized by extravascular lymphomatous infiltrations with (fig 4 *A*) or without (fig 4 *B*) leukostasis. In many chickens with lymphomatosis of strain 2 there were numerous gray lymphomas in the liver, measuring from 0.1 to 1 cm in the largest

TABLE 1—Blood Counts and Differential Counts on Chickens with Lymphomatosis of Strain 2

Chick kept*	Date of infection	Hemo- globin (Subl), per Cent	Red Cell Count, Thou- sands	White Cell Count, Thou- sands	Immature Red Cells			Lymphocytes†			Granulocytes			Throm- bo- cytes, per 100 White Cells	
					Polychrome		Basophil Prythro blasts	Mature, per Cent	Basophil		Poly- morpho nuclears, per Cent	Meta myelo- cytes, per Cent	Mast Cells, per Cent		
					Prythro cytes	Prythro blasts			Small, per Cent	Medium, per Cent					Large, per Cent
2275 Presutted spontane- ous lymphomatosis	March 28				Few	Few	0	74.0	2.5		23.5		1.0	16.0	2.15
	April 25		1,685	17.5	0	0	0	20.0	20.0	1.0	22.0			5.0	7.9
	April 27		1,198	130.0	0	0	0	3.0	15.0	1.0	4.0			3.0	3.8
2613 Inoculated April 28, died June 9	April 27				0	0	0	67.5	4.0	0.5	19.0		3.5	5.5	9.1
	May 19				Few	Few	0	71.0		2.0	14.0		1.0	12.0	8.5
	June 15	44	1,100	160.0	Many	Many	0	8.0	2.0	31.0	24.0		2.0	3.0	28
2770 Inoculated April 23, died May 18	March 21				0	0	0	66.0	1.0		29.0		0.5	3.5	10.4
	May 11	57	2,895	32.5	0	0	0	52.0	8.0	10.0	11.0		7.0	12.0	17.9
	May 18				0	0	0	19.0	11.0	28.0	15.0	1.0	2.0	12.0	10.9
2997 Inoculated Aug 11, died Oct 11	Aug 9				0	0	0	75.0	0.5		16.0	0.5	0.0	8.0	8.1
	Sept 17	15	1,940	63.0	Many	0	0	35.0	9.0	24.0	21.0	2.0	7.0	8.0	42
	Sept 20	36	2,195	51.4	Few	0	0	32.0	5.0	21.0	21.0	2.0	8.0	10.0	32
	Oct 14	38	1,325	35.0	Few	0	0	25.0	10.0	36.0	1.0	19.0	4.0	5.0	1.38
2976 Inoculated Aug. 6, died Oct 3	Aug 5				0	0	0	64.0			22.5			15.5	52
	Sept 17	36	1,680	24.5	Very many	Many	0	42.0	5.0	4.0	15.0	1.0	2.0	6.0	9
	Oct 3	35	1,430	16.5	Many	Few	0	63.0	1.0	21.0	2.0	3.0	5.0	6.0	56
3202 Inoculated Oct 27, died Nov 23	Nov 1				Few	0	0	64.0		1.0	17.5	3.0	4.5	10.0	41
	Nov 11				Few	Few	0	50.0	1.0	2.0	10.0		2.0	1.0	80
	Nov 23	20	995	98.5	Many	Many	Few	2.0	85.0	11.0			1.0	1.0	3

* Fowl 2255 is the chicken in which strain 2 originated.¹⁴ Chicken 2613 affords an example of lymphatic leukemia produced by transmission. In chicken 2770, there was a moderate increase of lymphocytes unassociated with anemia, and in chicken 2997, a similar increase of lymphocytes associated with anemia. In fowl 2976 the number of white cells in the blood was not increased, but many of the leukocytes were basophil lymphocytes of medium size, fowl 3202 is an example of lymphatic leukemia with severe anemia produced by a cell free virus.

† The differential counts are based on examination of dried smears containing 200 or more white cells. "Mature" designates small, not basophil, lymphocytes. Lymphocytes of an average diameter smaller than 9 microns are designated as "small," those measuring from 9 to 12 microns as "medium," and those larger than 12 microns as "large."

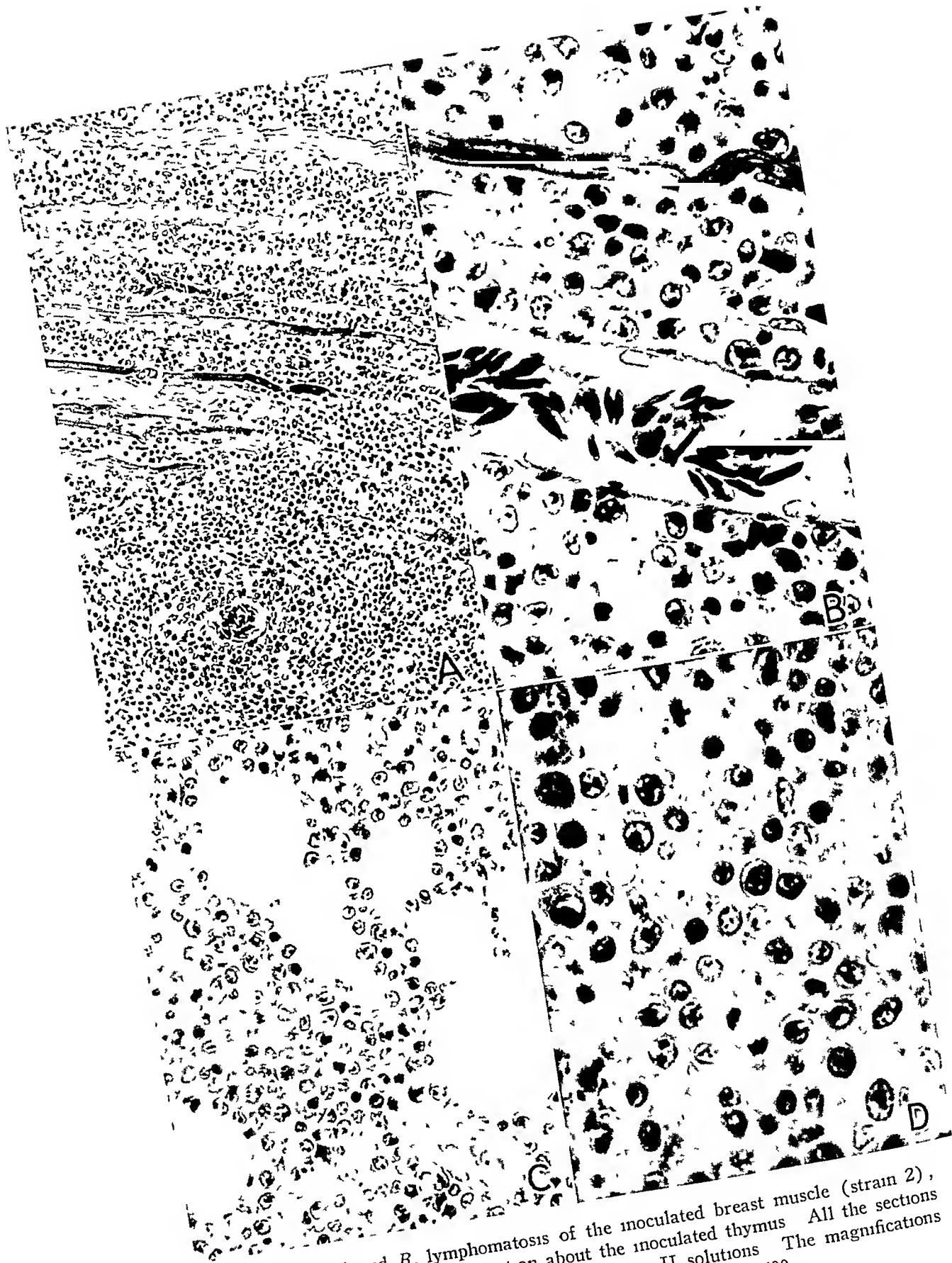


Fig 2—A and B, lymphomatosis of the inoculated breast muscle (strain 2), C and D, lymphomatous infiltration about the inoculated thymus. All the sections were stained with hematoxylin, eosin and azure II solutions. The magnifications are approximately A, $\times 150$, B, $\times 550$, C, $\times 350$, D, $\times 600$.

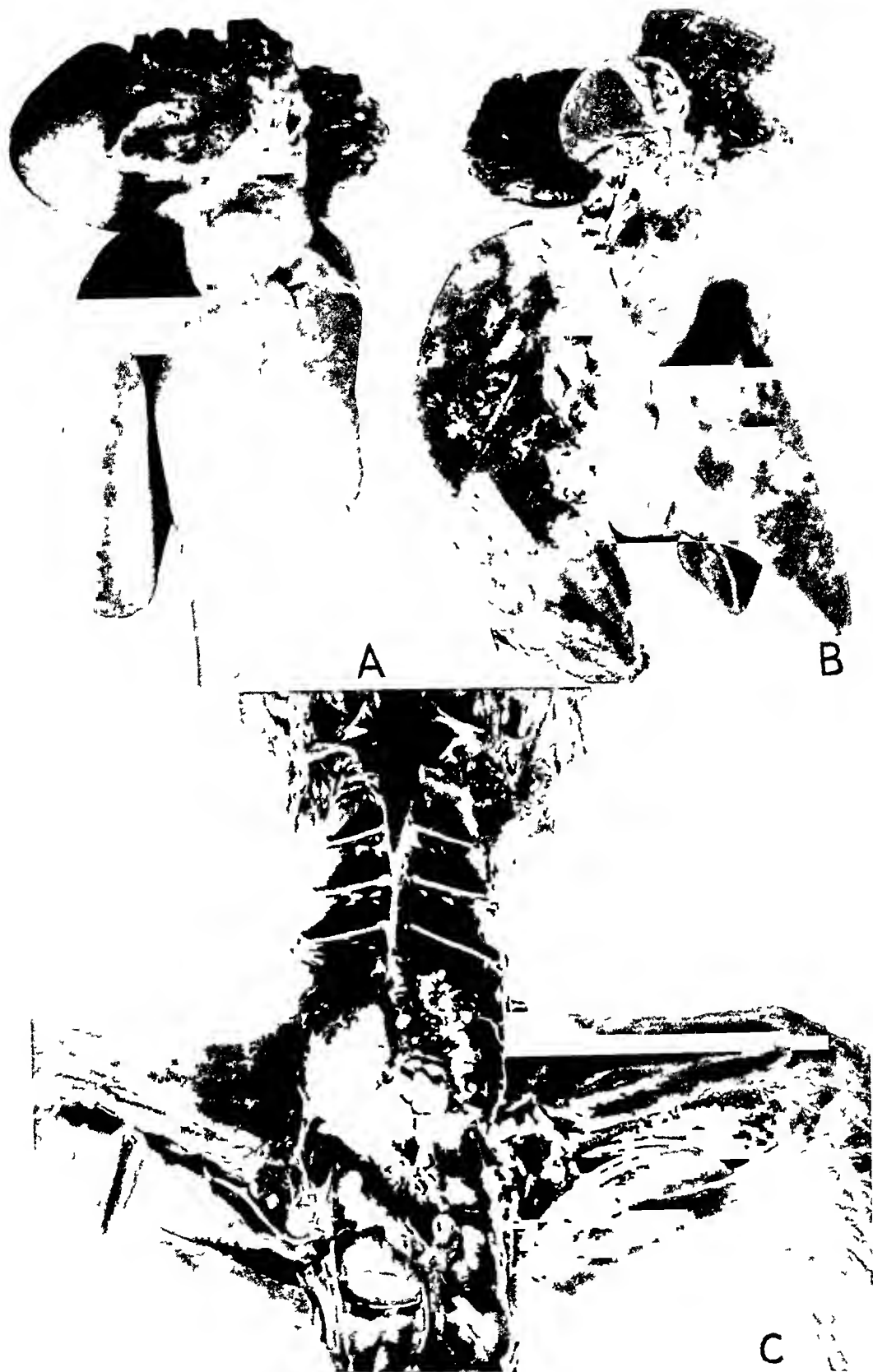


Fig 3—*A* and *B*, the liver, spleen, lung and heart of a chicken with lymphomatosis produced by strain 2. The liver shows, in *A*, minute gray spots, and in *B*, gray tumor nodules composed of large lymphocytes. Infiltration of the lung and enlargement of the spleen are shown in *A*. *C* shows tumor-like thickening of the inoculated right sciatic nerve associated with extensive infiltration of all lobes of the right kidney (strain 2).

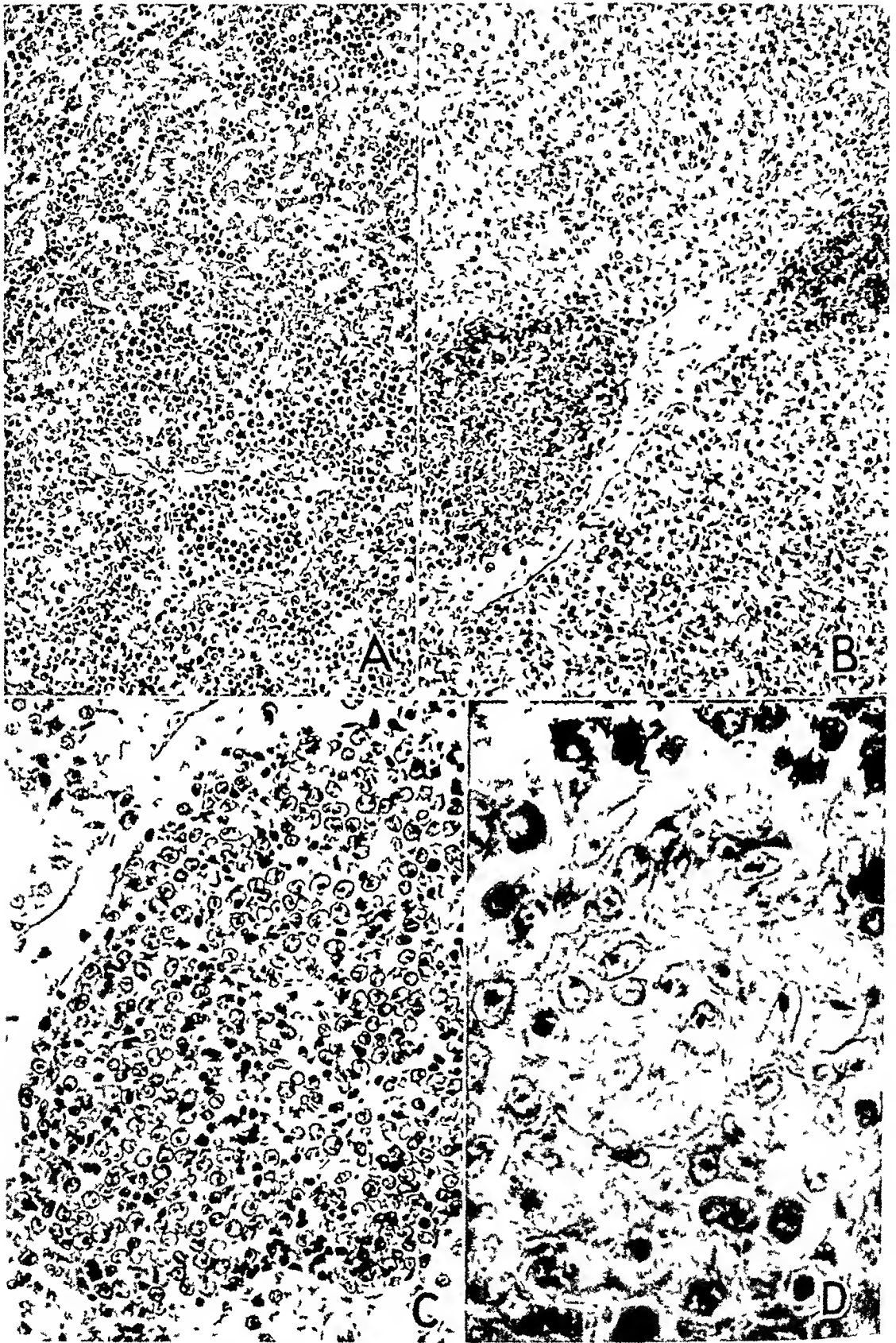


Fig 4—Lesions in the liver produced by strain 2. *A* shows perivascular and diffuse infiltrations. *B* and *C* show tumor nodules composed of cells many of which are probably endothelial or reticular. *D* shows a whorl-like arrangement of similar cells. The sections were stained with eosin and azure II solutions. The magnifications are approximately: *A* and *B*, $\times 180$, *C*, $\times 400$, *D*, $\times 700$.

diameter (fig 3 *A* and *B*) In a few chickens the extravascular infiltration was slight and the liver appeared normal on gross examination The presence of large numbers of basophil lymphocytes in the blood vessels distinguishes this disease from spontaneous lymphomatosis associated with great enlargement of the liver ("big liver" disease, hepatolymphomatosis [fig 5 *A* and *B*]) In most instances of hepatolymphomatosis the bone marrow and spleen appear grossly normal The few instances of this type of lymphomatosis occurring among the passages of strain 2 may have been spontaneous Yet in at least one instance of lymphomatosis the autopsy observations were indistinguishable from those of spontaneous hepatolymphomatosis, although basophil lymphocytes were seen in the blood ante mortem

The lymphomatous infiltrations in the kidney heart and lung were similar to those of the liver, either affecting the organ diffusely or producing tumor nodules up to about 1 cm in diameter In the spleen the infiltration was localized in the follicles as well as in the pulp Since the pulp also contained numerous erythrocytes it looked grayish red, while the follicles were gray, hence the gray mottling on gross examination This organ varied in size from approximately normal to about ten times normal The bone marrow was gray-red, "pyoid"

Considerable difficulty was often met with in distinguishing the large basophil lymphocytes of strain 2 from endothelial cells and basophil erythroblasts (figs 4 *C* and 6 *A* and *C*) The large clear cells shown in these figures resemble closely those produced by the endothelioma virus of Begg and Murray,⁶ but that virus does not produce lymphomatosis

The frequent association of endothelial neoplasms with lymphomatosis is characteristic of strain 2, for it is not present in neurolymphomatosis⁷ and in other varieties of lymphomatosis of chickens⁸ The histologic characteristics of endothelioma produced by this strain have been described⁹ Growth of cells, probably endothelial, occurring in association with lymphomatosis is shown in figures 4 *C* and *D* and 6 *A* and *C* The virus of strain 2 also stimulates the growth of myelocytes¹⁰ and it has been suggested that the large basophil lymphocytes of strain 2 function as hemocytoblasts in the sense of Maximow¹

6 Begg, A M, and Murray, J A. *Scient Rep Invest Imp Cancer Research Fund* 9 1 1930

7 Pappenheimer, A M, Dunn L C and Seidlin S M. *J Exper Med* 49 87 1929

8 (a) Tyzzer E E and Ordway T J M. *Research* 21 459, 1909 (b) Mathews F P and Walkey, F L. *J Cancer Research* 13 383, 1929

9 Furth, J. *J Exper Med* 59 501, 1934

10 Furth, J.

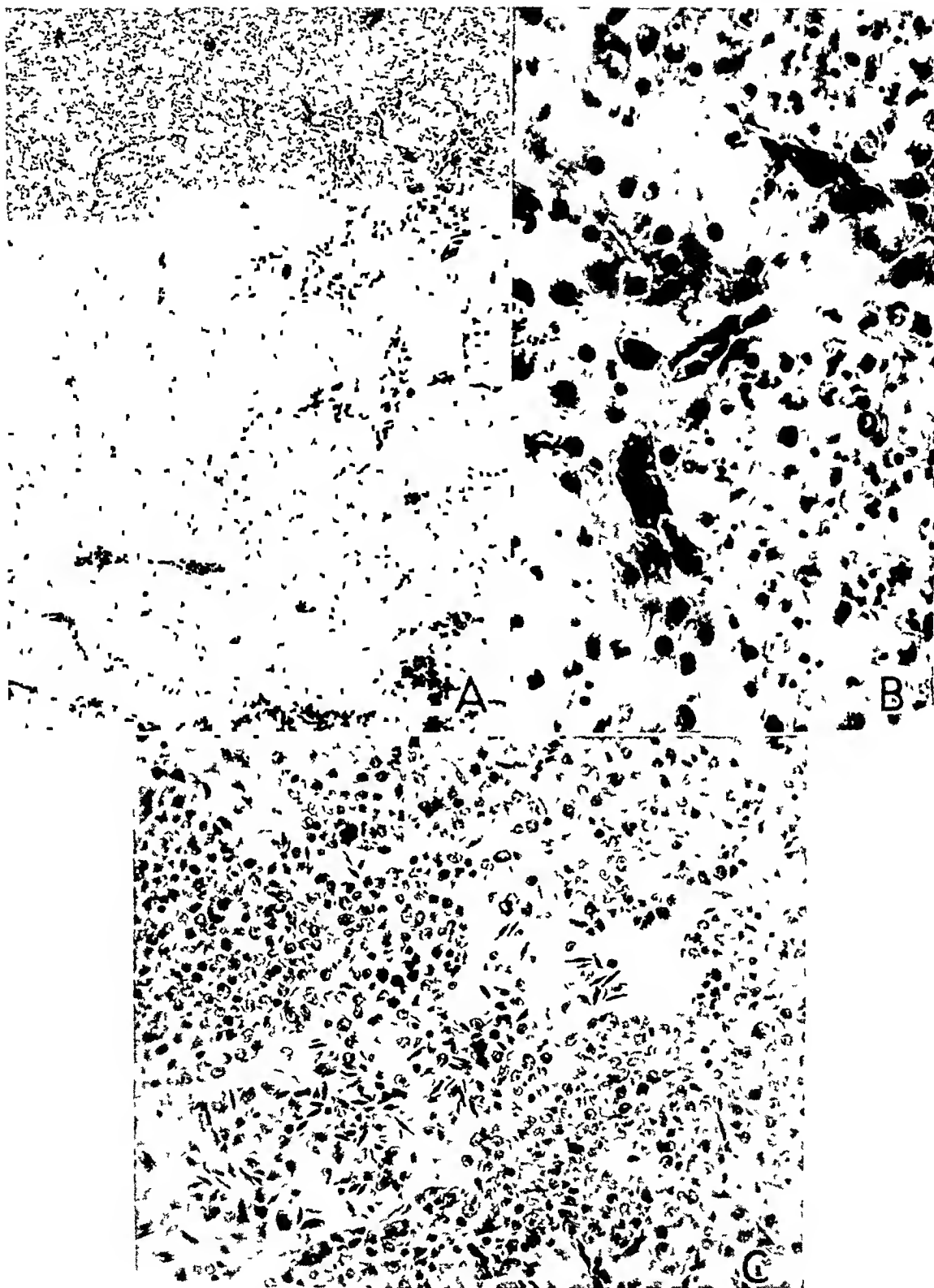


Fig 5—*A*, spontaneous hepatolymphomatosis ("big liver"), $\times 60$ *B*, spontaneous hepatolymphomatosis with higher magnification, karyorrhexis of malignant lymphocytes, $\times 450$ *C*, lymphomatous infiltration in the liver of a chicken with spontaneous neurolymphomatosis, $\times 300$ The sections were stained with eosin and azure II solutions The magnifications given are approximate

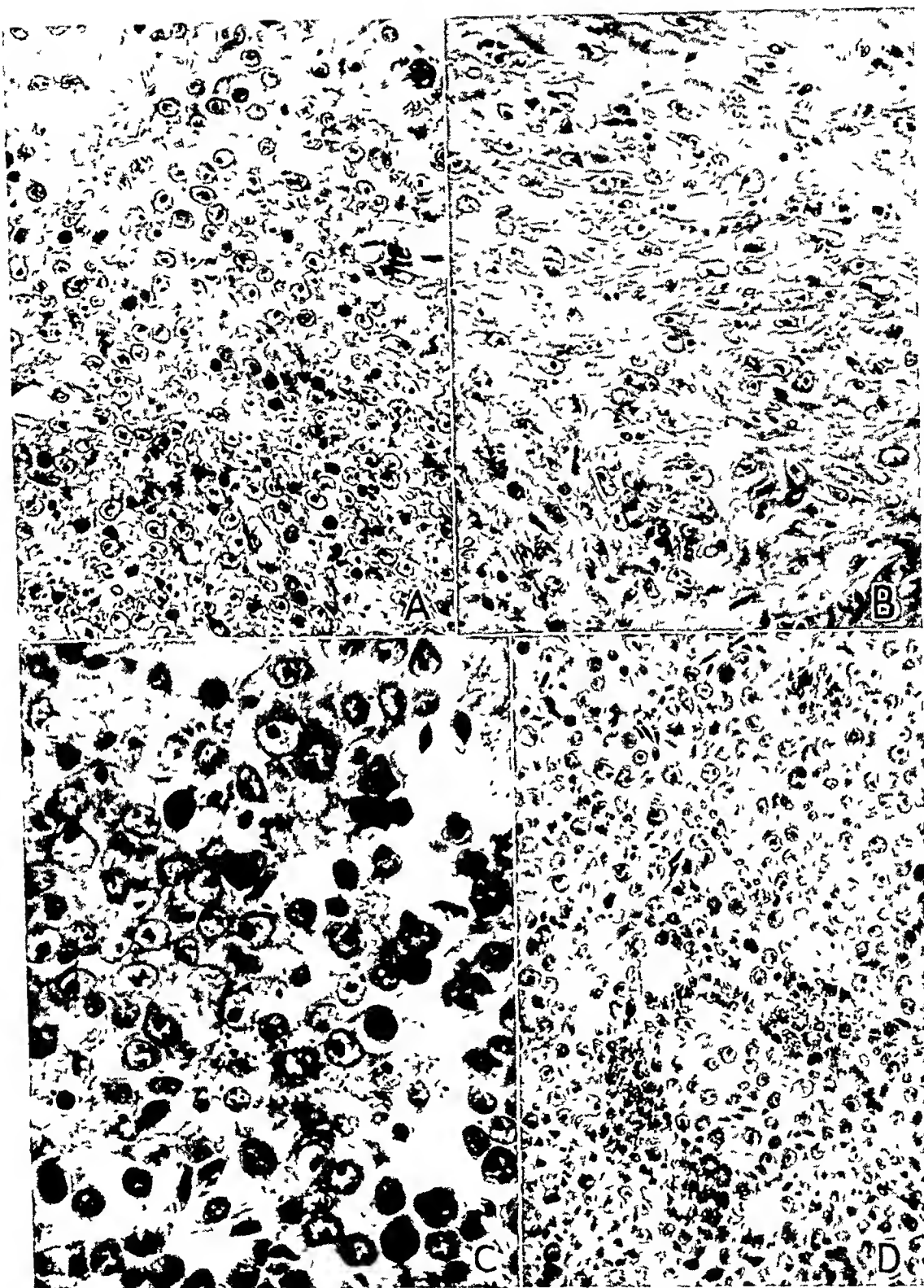


Fig 6—*A*, proliferation of cells, probably endothelial, in the breast muscle inoculated with strain 2, $\times 400$ *B*, sarcoma-like growth in the inoculated breast muscle, $\times 450$ *C*, growth in the kidney similar to that shown in *A*, $\times 700$ *D*, hyperplasia of the bone marrow, $\times 350$ *A*, *B*, *C* and *D* are from chickens inoculated with lymphomatosis strain 2. The sections were stained with eosin and azure II solutions. The magnifications given are approximate.

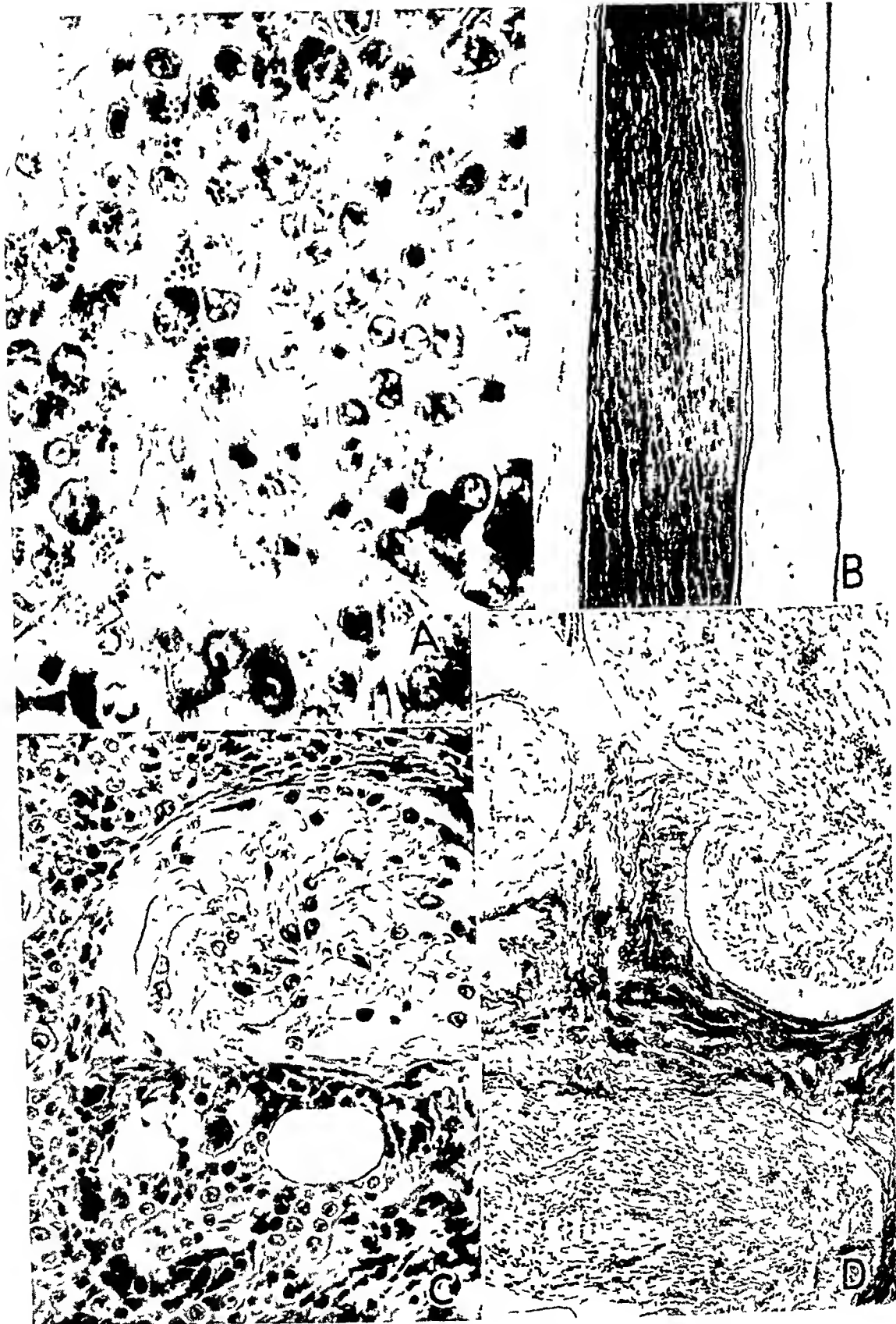


Fig 7—*A*, formation of myelocytes in the portal area of the liver, $\times 800$ *B*, diffuse lymphomatous infiltration of one bundle of fibers of the sciatic nerve, adjacent nerve bundles are almost free from infiltration, $\times 20$ *C*, infiltration of the vagus and surrounding tissue following intrathymic inoculation, $\times 400$ *D*, infiltration of the brachial plexus following intravenous inoculation, $\times 80$ *A*, *B*, *C* and *D* are from chickens with lymphomatosis of strain 2. The sections were stained with eosin and azure II solutions. The magnifications given are approximate.

Proliferation of myelocytes in association with hemocytoblasts is shown in figure 7 *A*. The predominating type of large round cells that infiltrated the marrow (fig 6 *D*) could not be distinguished microscopically from basophil erythroblasts. However, erythroblasts seldom produce extravascular infiltration, and they do not infiltrate ganglions and nerves, even after intraneural introduction (see p 38). Furthermore, in blood smears basophil lymphocytes can be readily distinguished from erythroblasts (fig 1 *D*). Lymphomatous infiltration of nerves, a frequent finding among chickens given injections of strain 2, is illustrated in fig 7 *B*, *C* and *D*. Post mortem large lymphocytes rapidly undergo pyknosis and karyorrhexis, repeated observations suggest that these changes in the lymphocytes precede similar changes in other cells of the organs which they infiltrate (e g liver cells, erythrocytes [fig 5 *A*]).

INTRAMUSCULAR TRANSMISSION WITH CELL-CONTAINING MATERIAL

The endothelioma of Begg and Murray,⁶ all sarcomas of Rous¹¹ and the similar neoplasms studied in England under the Imperial Cancer Research Fund¹² readily produce tumors in the breast muscle. On the contrary, Ellermann and Bang,^{1a} Jármai,¹³ Engelbreth-Holm and Rothe Meyer¹⁴ and other workers, including ourselves, found no tumor formation at the site of inoculation when material from the common type of transmissible leukosis (like our strain 1) was injected into the muscle and subcutaneous tissue.

Oberling and Guérin¹⁴ suggested that our failure to discover the ability of the virus of Ellermann to produce tumors was due to the fact that we used the intravenous route for inoculation. We have made numerous unsuccessful attempts to produce tumors with leukotic tissues of strain 1 by inoculations other than intravenous.¹⁵ In some of these experiments leukotic cells of strain 1 were embedded in plasma clot preceding the inoculation in order to facilitate tumor formation at the site of inoculation. Since the percentage of successful inoculations was smaller after subcutaneous and intramuscular injections than after intravenous injections, we decided to transfer strain 1 by the intravenous route only. The observations of Oberling and Guérin induced us to test again whether leukosis of strain 1 produced tumors in the inoculated thymus and breast muscle, and we found that it

11 Rous, P. *J. A. M. A.* **56** 198, 1911. Rous, P., Murphy, James, and Tytler, W. H. *ibid.* **59** 1793, 1912. Rous, P., and Lange, L. B. *J. Exper. Med.* **18** 651, 1913.

12 Foulds, L. *Scient. Rep. Invest. Imp. Cancer Research Fund* **11** 1, 1934.

13 Jármai, K. *Arch. f. Wissensch. u. prakt. Tierh.* **62** 113, 1930, **65** 46, 1932.

14 Oberling, C. and Guérin, M. *Bull. Assoc. franç. etude du cancer* **20** 180 and 326, 1933.

15 Furth¹¹, Stubbs, E. L., and Furth, J. Unpublished work.

did not. The characteristics of this strain are the same now as they were four years ago,³⁷ and it still produces erythroleukosis and myeloblastic leukemia but does not produce endothelioma or Rous sarcoma.

Leukosis strain 2, in contrast with the common varieties of leukosis strains, produces extensive extravascular infiltration or tumors composed of myelocytes or cells like large lymphocytes, it also produces endothelioma. It may therefore be supposed that inoculation with cells of strain 2 would produce lymphomatosis, myelomatosis or endothelial growth in the breast muscle, depending mainly on the type of cell introduced.

Series 1—The first series of intramuscular inoculations, reviewed in a former report,^{1a} were unsuccessful. In these experiments, made between April and July 1932, twenty-nine chickens were inoculated intramuscularly, and no tumors developed at the sites of injection. One chicken died of general lymphomatosis, ten died of intercurrent disease within one or two months after inoculation, and eighteen remained healthy.

Series 2—Seven months later further attempts were made to produce tumors in the thymus, where spontaneous leukotic tumors of all kinds are often seen, and in the breast muscle, in which Rous sarcoma and endothelioma grow readily.

In ten experiments, thirty chickens were given injections of blood cells or tumor tissue composed of large lymphocytes, myelocytes or neoplastic endothelium. Most of the injections were made into the breast muscle, subcutaneous tissue or thymus, and a few into the region of the kidney. Leukosis developed in twenty-four (80 per cent) of the inoculated birds, and in twelve of them grossly visible infiltrations or tumors were found in the muscle or thymus at the site of inoculation. In two chickens there was in addition, a sarcoma-like growth (fig 6 B) which, although it had the character of a malignant growth, remained small barely detectable with the naked eye, and limited to the site of injection. The last-mentioned characteristics distinguish it from Rous sarcoma, which, even if mixed with the agent of leukosis, grows profusely in the breast muscle receiving the injection and often metastasizes to distant organs. In seven of these experiments the material used for intramuscular injection was also injected intravenously, and leukosis developed in twelve (63 per cent) of nineteen chickens thus inoculated.

The first of these experiments, made with cells of a tumor composed mainly of myelocytes, had the following result. Three of the four birds given intramuscular injections acquired systemic leukosis, chiefly lymphomatosis, and in one of these there was extensive infiltration of the inoculated breast muscle by cells like large lymphocytes (fig 2 A and B). In another experiment two of the three

chickens given injections of a cell suspension of myelocytoma associated with endothelioma presented infiltration of the inoculated thymus (fig 2 *C* and *D*) and breast muscle by large lymphocytes. The vast number of mitoses (as many as 15 in a field magnified 400 times) gave evidence of the unrestricted growth of the large lymphocytes of strain 2. Since the blood-forming organs of one of these two birds showed only slight alterations, and mitosis among the lymphocytes was abundant at the site of injection, it is probable that these cells grew unrestrictedly in the inoculated thymus and breast muscle. It is noteworthy that the tumor tissue injected, composed mainly of myelocytes and endothelial tumor cells, contained few large lymphocytes.

The largest tumor that developed in a thymus lobe receiving an injection measured 3 by 2.5 by 2 cm. fifty-two days after inoculation, when the bird was killed for study. Thymus lobes not receiving injections and the liver, spleen and bone marrow were normal. These observations further support the view that the tumor at the site of inoculation originated in the implanted neoplastic cells.

In another experiment minced tissue of an ovarian tumor (endothelioma with giant cells) mixed with minced tissue of a hepatic tumor (lymphoma) of the same bird was injected into both the right breast and a lobe of the thymus in six chickens. Two of these were killed four days and another two were killed ten days after inoculation for the purpose of studying the early lesions. Four and ten days after inoculation the alterations were localized to the sites of injection and consisted of apparently neoplastic growth of large lymphocytes, these cells evidently originated from those introduced. In two chickens examined thirty-one and thirty-eight days after inoculation small sarcoma-like growths (fig 6 *B*) were found at the sites of injection. These growths remained localized, and the distant lesions were those of leukosis, mainly lymphomatosis. The significance of these sarcoma-like lesions remains obscure. They were never seen again among the large number of birds successfully inoculated in a similar manner.

These experiments have shown that lymphomatosis may be produced in the breast muscle with leukosis strain 2.

Series 3—After these partially successful attempts we succeeded in producing readily transmissible lymphomatous tumors in the breast muscle (fig 8). Lymphomatous tumors composed of large lymphocytes developed in almost every inoculated bird. This was followed by invasion of the blood (lymphatic leukemia) and metastatic infiltration in numerous organs, mainly the bone marrow, liver, spleen, ovary, peripheral nerves and ganglions.

Chicken 3870 (strain 2) had numerous lymphomatous tumors in the liver and kidney. A suspension of particles of these tumors was injected into the thymus and breast muscle in three chickens, two of which acquired lymphomatous tumors at the sites of injection and died with blood changes of leukemia associated with lymphomas of numerous organs. The results of the subpassages are shown in figure 8. The microscopic changes in chicken 4339 were suggestive of neuro-lymphomatosis (see p 23).

Summary—Strain 2 transmitted intramuscularly with material containing lymphocytes produces lymphomatous infiltration or tumors in the muscles receiving injections. The following observations indicate

that these neoplasms are the result of unrestricted multiplication of the large lymphocytes that were introduced. Mitosis is abundant among the large lymphocytes, proliferative changes in normal cells are absent at the site of injection, the lymphocytes at the site of infiltration have the same cytologic characteristics as those introduced, the blood-forming organs of chickens with lymphomatous tumors in the inoculated breast muscle show no alterations at the early stage of the disease.

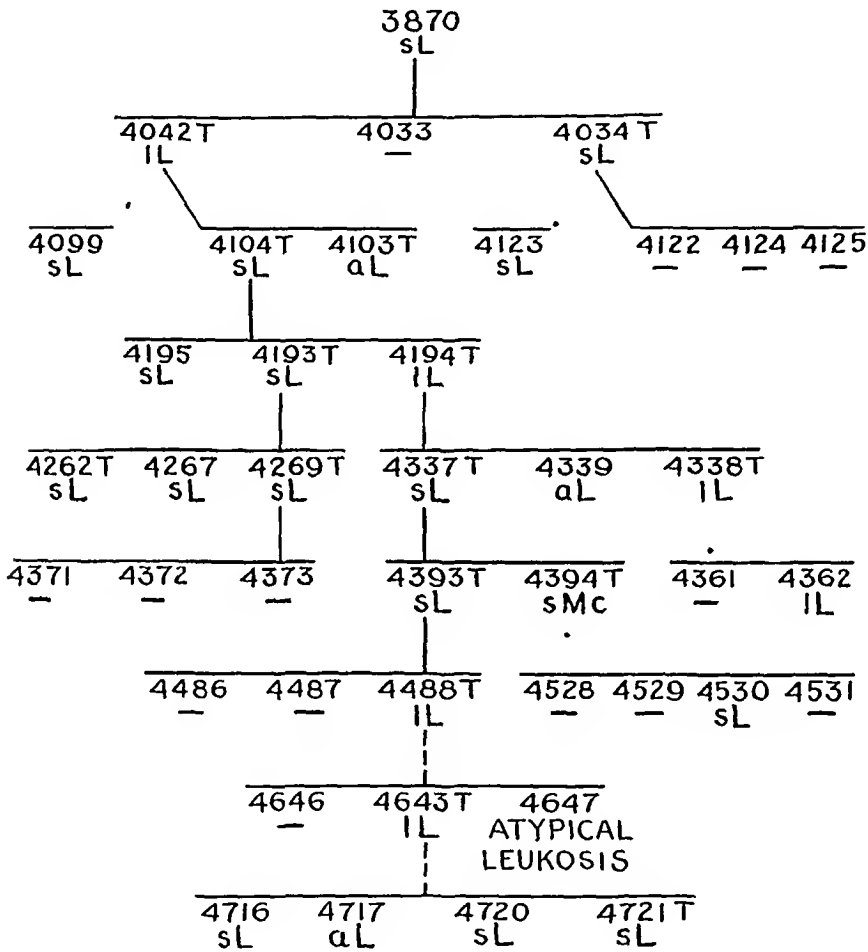


Fig 8—Transmission experiments with lymphomatosis strain 2. Each number is that of a chicken. A vertical solid line indicates intramuscular injection, a broken vertical line, intramuscular and intravenous injections, and a dotted line, intravenous injection. *sL* signifies subleukemic lymphomatosis, *T*, tumor in the inoculated breast muscle, *IL*, leukemic lymphomatosis, *aL*, aleukemic lymphomatosis, *sMc*, subleukemic myelocytomatosis, —, inoculation unsuccessful.

ATTEMPTS AT INTRAMUSCULAR TRANSMISSION WITH CELL FREE VIRUS

The experiments described in the preceding section show that the large lymphocytes of strain 2 behave like malignant cells, undergo unrestricted multiplication at the site of introduction, invade the blood

stream, and produce metastases. They differ from neoplastic cells in mammals in that they elaborate a filtrable agent that transforms normal lymphocytes into malignant ones. The following experiments show that the cell-free virus of strain 2, in contrast to the virus of Rous sarcomas and that of the endothelioma of Begg and Murray, does not produce neoplasms in muscles receiving injections. The chickens given intramuscular injections of cell-free material either remained healthy or acquired the systemic disease with no infiltration at the site of injection.

Cell-free material of lymphomatous chickens was tested and in no instance produced a tumor at the site of injection. In most experiments dried blood or tumor tissue was used for the injection, in two experiments dried plasma and in one frozen and thawed blood were used. In seven of the eight instances of leukosis produced by the cell-free virus large lymphocytes were numerous in the blood. One chicken had leukemic myelocytomatosis.

TABLE 2—*Data on Injections of Cell-Free Virus of Strain 2*

Material Injected	Route of Injection*	Chickens Inoculated	Chickens with Leukosis
Cell free	Intrav and Intram	29	4
Cell free	Intrav	4	1
Cell free, repeated injections with material from several chickens	Intrav and intram	6	3
Cell containing	Intrav	13	9
Cell containing	Intrav and intram	4	4

* "Intrav " and "intram " are abbreviations for intravenous and intramuscular

Summary—The virus of strain 2 must reach blood-forming tissues in order to produce leukosis. It does not produce tumors in the muscle tissue receiving the injection.

INTRANEURAL TRANSMISSION

Strain 2 has an affinity for nerve tissues⁴ⁿ and, as the following experiments show, is readily transmitted by intraneural inoculations.

Experiment 1—Intraneural injection of lymphomatous tumor tissue gave neurolymphomatosis in four chickens that died or were killed and examined at twenty-five, thirty, thirty-two and thirty-seven days, respectively, after inoculation (average, killed or died at thirty days).

Experiment 2—Intraneural injection of tumor tissue and intravenous injection of 0.1 cc of blood gave neurolymphomatosis in one chicken that died and was examined at twenty-five days.

Experiment 3—Intramuscular injection of lymphomatous tumor tissue and intravenous injection of 0.1 cc of blood gave neurolymphomatosis in four chickens that were killed or died and were examined at twenty-seven, thirty-two, forty-two and forty-nine days, respectively, after inoculation (average, killed or died at thirty-seven and a half days).

In these three experiments all inoculations were successful.

The experiments show that strain 2 can be readily transmitted by intraneural injection. The inoculated nerves became greatly thickened, and there was extensive lymphomatous infiltration about the nerve extending by continuity into the adjacent muscle tissue (fig 3 C). Subsequently, large basophil lymphocytes and erythroblasts appeared in the blood.

Another experiment with fewer animals yielded similar results.

The neurolymphomatosis strains 5 and 6 that will be described in part II do not produce anemia and are seldom associated with lymphatic leukemia.

Summary—The intraneural route is very favorable for the transmission of lymphomatosis strain 2, and the large lymphocytes of strain 2 proliferate in and about the inoculated nerves.

II NEUROLYMPHOMATOSIS (FOWL PARALYSIS) PRODUCED BY STRAINS 5 AND 6

In 1907 Marek,¹⁶ in Hungary, described a disease characterized by extensive round cell infiltration of the peripheral nerves and of the posterior root ganglions and named it "neuritis interstitialis." Two types of nontraumatic paralysis have been since recognized in the bird: (a) nutritional paralysis due to noninflammatory, degenerative changes caused by vitamin B deficiency (Eijkmann) and (b) Marek's *Geflügel-lähme*.

"Infectious polyneuritis," "neuromyelitis," "range paralysis," "fowl paralysis," and "neurolymphomatosis" are synonymous terms proposed for Marek's paralysis, of which only the term "neurolymphomatosis" will be used in this report. Neurolymphomatosis has been observed in the United States and Canada, in several countries of Europe, in Africa and in Japan.¹⁷ It is now recognized as one of the most common fatal diseases of young chickens from 3 to 11 months of age.

Failure of experimental transmission of the disease has been reported by numerous workers, although most of them have expressed the belief that the causative agent is a virus.

Successful transmission of neurolymphomatosis was first described by Van der Walle and Winkler-Junius, but the evidence presented by these, as well as by several other workers, is not sufficient to support their conclusions.⁵ The first experiments that strongly suggested the transmissibility of neurolymphomatosis were made by Pappenheimer, Dunn and Seidlin.⁷ Paralysis occurred in 26 per cent of their experimental birds as compared with 8 per cent of their uninoculated controls.

¹⁶ Marek, J. *Deutsche tierarztl. Wchnschr.* **15** 417, 1907.

¹⁷ The literature on neurolymphomatosis has recently been reviewed by Jungherr, E. *Storrs Agric. Exper. Stat. Bull.*, no 200, 1934.

Dalling and Warrack¹⁸ observed that 27 per cent of the inoculated chickens acquired paralysis, as compared with 2.5 per cent of the uninoculated controls. It is noteworthy, however, that the death rate, due mainly to coccidiosis, was 38.5 per cent among their inoculated chickens and only 3.8 per cent among their uninoculated controls, but this is obviously no evidence that coccidiosis was influenced by the inoculations. Transmission experiments of Seifried¹⁹ made on a small number of chickens suggested that neurolymphomatosis is transmissible by feeding, but this has not been confirmed.

The etiology of neurolymphomatosis and its relation to leukosis of chickens are subjects of much controversy.

Ellermann and Bang and most workers who subsequently studied leukosis of chickens did not observe paralysis among their experimental birds, most of those who studied neurolymphomatosis, on the contrary, noted the frequent association of fowl paralysis with lymphomatous tumors of the viscera.⁷ Our experiments have shown that there are several agents that produce leukosis, one (that of strain 1) causes erythroleukosis and myeloid leukosis and is unassociated with infiltration in the nervous system, while another (that of strain 2) produces lymphomatosis with infiltration in the nervous system. Strain 2 almost invariably causes severe anemia, extensive lymphomatous infiltration of the bone marrow and the appearance of numerous large basophil lymphocytes in the blood, but none of these alterations are known to occur in association with neurolymphomatosis. These differences have suggested that the causative agent of fowl paralysis is not identical with any of the known strains of leukosis, and this suggestion is confirmed by the experiments described here.

Although several workers found a greater incidence of paralysis among their experimental birds than among uninoculated controls, none described a strain that could be passed readily from diseased to healthy chickens and studied in successive passages. Since the filtrability of some strains of avian tumors and of avian leukosis is demonstrated with difficulty, experiments to demonstrate the filtrable cause of the disease were not undertaken until the disease had been transmitted by viable cells. We have succeeded in isolating strains of neurolymphomatosis with the technic used for grafting malignant cells of mammals. The disease could not be transmitted with cell-free material in spite of the ease with which it was transmitted by material containing viable lymphocytes. In transmission experiments neurolymphomatosis behaved like a neoplasm in which the malignant cells are lymphocytes with special affinity for the peripheral nerves.

¹⁸ Dalling, T., and Warrack, G. H. *Atti de V Congresso Mondiale di Pollicoltura*, 1933, no. 90.

¹⁹ Seifried, O. *Arch. f. wissensch. u. prakt. Tierh.* **62**: 209, 1930.

MATERIAL OF STUDY AND PROCEDURES

All chickens observed with spontaneous neurolymphomatosis and most chickens used in the experimental work were Barred Rocks, also a few White Leghorns tested were found to be susceptible to the disease²⁰ The experimental birds were from 4 to 15 weeks of age when first given injections

Infiltrated nerves and lymphomatous tumors were cut up in Locke's or Tyrode's solution and filtered through a small piece of cotton for intraneural and intravenous inoculation Intraneural injections were made in the exposed sciatic or ulnar nerves of anesthetized birds The whole blood used for injection contained approximately one tenth of its volume of heparin solution (0.1 per cent) The amount injected was estimated to be approximately from 0.01 to 0.02 cc

At the beginning of the experiments only those birds that showed gross evidence of neurolymphomatosis were studied microscopically, so that several of the chickens given in this report as not showing neurolymphomatosis may have had microscopic lesions of the disease Microscopic examination of nerves often disclosed unquestionable neurolymphomatosis that was not detected on gross examination Since the location of infiltration varied greatly and usually only from two to five nerves and the sympathetic ganglion near the adrenal were taken for microscopic examination, several cases of neurolymphomatosis may have escaped detection

The incidence of death due to conditions other than fowl paralysis was high among the experimental birds Most of the birds that appeared healthy and were killed five months after inoculation when the experiment was brought to an end were not examined microscopically Some chickens were killed in the terminal stage of paralysis, and a smaller number were killed to combat small spontaneous epidemics, such as fowl pox, coryza or coccidiosis

BLOOD CHANGES

Most workers have failed to observe blood changes in association with neurolymphomatosis, but Johnson²¹ reported an increase of monocytes and mast cells and the presence of 'budding lymphocytes' in the blood

With rare exceptions the blood of our chickens with neurolymphomatosis appeared normal during the entire course of illness There was a moderate or great increase of lymphocytes in the blood of chicken 3878 in which strain 5 originated and in chickens 3831 and 4570 inoculated with this strain (table 3) Almost all of these lymphocytes were small, but many of them were basophil (fig 9 B and C) Lymphatic leukemia occurred in a fourth chicken (3948) on invasion of the blood by basophil lymphocytes of medium and large size (fig 9 D and E) It is possible that this was a spontaneous disease Erythroblasts were never found in the blood of chickens with neurolymphomatosis All three chickens with small cell lymphatic leukemia had extensive neural and visceral lymphomatosis

Differential counts were made on the blood of ten chickens with transmitted lymphomatosis the blood of which appeared normal on routine

²⁰ The spontaneous disease is known to affect all breeds of chickens

²¹ Johnson, E. P. Virginia Agric Exper Stat Bull, no 44, 1932 1934, no 56, J Am Vet M A 83 325, 1933

TABLE 3—*Differential Counts on the Blood of Chickens with Neurolymphomatosis*

Chicken	Date of Examination	White Cell Count	Lymphocytes*				Granulocytes			Thrombocytes per 100 White Cells
			Mature, per Cent	Basophil		Large, per Cent	Poly morpho nuclears, per Cent	Meta myelo cytes, per Cent	Mast Cells, per Cent	Mono cytes, per Cent
				Small, per Cent	Medium, per Cent					
3578 Presented spontaneous neurolymphomatosis	July 11	145,000	89.0	4.0	2.0		5.0			0.5
3831 Inoculated July 18, died Sept 15	June 30	Normal	38.0				35.0		1.5	5.5
	Aug 22	Moderate increase	11.0	11.5			35.0	21.0	5.0	16.5
	Sept 15	Moderate increase	35.0	45.0			2.5	12.0	0.5	5.0
4570 Inoculated May 28 died June 28	March 27	Normal	54.0	3.0			34.0	2.0	3.0	4.0
	June 28	Moderate increase	35.0	54.0	2.0		7.0		1.5	0.5
3948 Inoculated Sept 21, died Nov 17	Sept 22	Moderate increase	29.0	1.0			58.0		3.0	9.0
	Oct 23	Moderate increase	18.0	8.5			42.0	6.0		25.5
	Nov 17	Great increase	1.0	15.0	46.0	13.0	9.0	2.5	0.5	13.0
Average of ten chickens Before inoculation			61.5	5.0			22.7	0.3	3.2	7.3
At height of illness			31.3	14.3	0.3	0.1	38.0	2.0	2.7	11.3

* For explanation of terms, see table 1

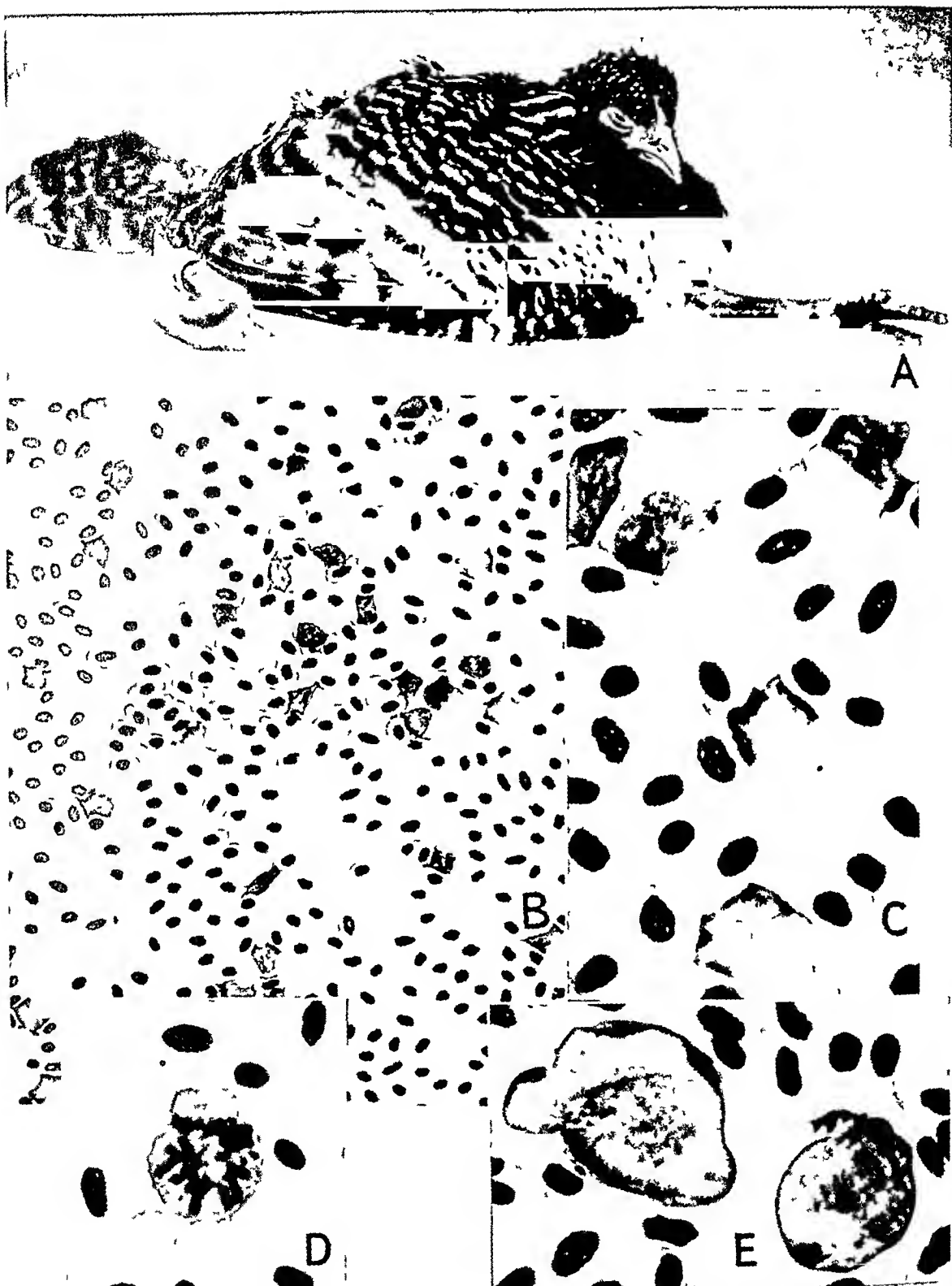


Fig 9—*A*, characteristic posture of a fowl with paralysis of the legs *B*, lymphoid leukemia in a fowl (3878) with spontaneous neurolymphomatosis, $\times 400$ *C*, higher magnification of the lymphocytes shown in *B*, $\times 1100$ *D*, a mitotic figure *E*, two lymphocytes from the blood smear of a fowl with large cell lymphoid leukemia (3948, strain 5), $\times 1100$ The blood smears were stained with Wright and Giemsa solutions The magnifications given are approximate

examination Eight showed a conspicuous increase in the percentage of small basophil lymphocytes, the average figure for these cells rose from 5 per cent before inoculation to 14.3 per cent shortly before death (table 3) The small basophil lymphocytes in the blood of paralyzed chickens were indistinguishable from the similar cells of normal chicken blood

ANATOMIC CHANGES

The anatomic changes associated with neurolymphomatosis have been well described by numerous workers We refer particularly to the articles of Marek¹⁶ and Pappenheimer, Dunn and Cone⁵ and mention only observations that deviate from or add to those of previous workers The changes produced by strains 5 and 6 are indistinguishable from those associated with the spontaneous disease The characteristic posture of a chicken with neurolymphomatosis of the sciatic nerves and lumbosacral plexuses is shown in figure 9 *A*

The basic alteration in neurolymphomatosis consists of infiltration of the peripheral nerves (fig 10 *B*, *C* and *D*) and ganglions (fig 11 *A* and *B*) by cells like lymphocytes The character of the infiltration is in most instances that of a lymphomatous neoplasm The degenerative and inflammatory changes with which lymphomatosis is associated may be regarded as secondary Several nerves are usually involved but to a variable extent, almost any peripheral nerve may be involved, the vagus (fig 12 *A*), the brachial plexus (fig 12 *D*), the intercostal, splanchnic and lumbosacral plexuses (fig 12 *B*) and their branches are common sites of neurolymphomatosis The diagnosis can be made in the majority of instances from the thickening, grayish discoloration and loss of the normal cross-striations of nerves In a small number of instances the changes are scant, and the disease may be overlooked on gross examination Since microscopic examination of the entire peripheral nervous system is not feasible, it is impossible to exclude the presence of mild lesions of neurolymphomatosis in any bird The lymphocytes are of small or medium size, like normal lymphocytes in appearance

Usually the small, occasionally the medium-sized, lymphocytes are predominant Among the lymphocytes of medium size mitosis is abundant The absence of polymorphonuclear leukocytes is significant The lymphocytes are found scattered diffusely between the nerve fibers, but often there is a conspicuous perivascular cuffing in and about the nerve The diagnosis of fully developed neurolymphomatosis offers no difficulties, the meaning of mild lymphocytic infiltration is doubtful Lymphoid infiltration, not neoplastic in character, is often associated with the formation of true germinal centers (fig 13 *C* and *D*) showing histologic evidence of lymphocyte formation (fig 13 *D*) Formation of germinal centers, absent in lymphomatosis, may be regarded as evidence of orderly lymphocyte formation



Fig 10—A, thickening of the inoculated sciatic nerve (strain 5) B, C and D, infiltration of nerves in spontaneous neurolymphomatosis The sections were stained with eosin and azure II solutions The magnifications are approximately B, $\times 450$, C, $\times 60$, D, $\times 250$

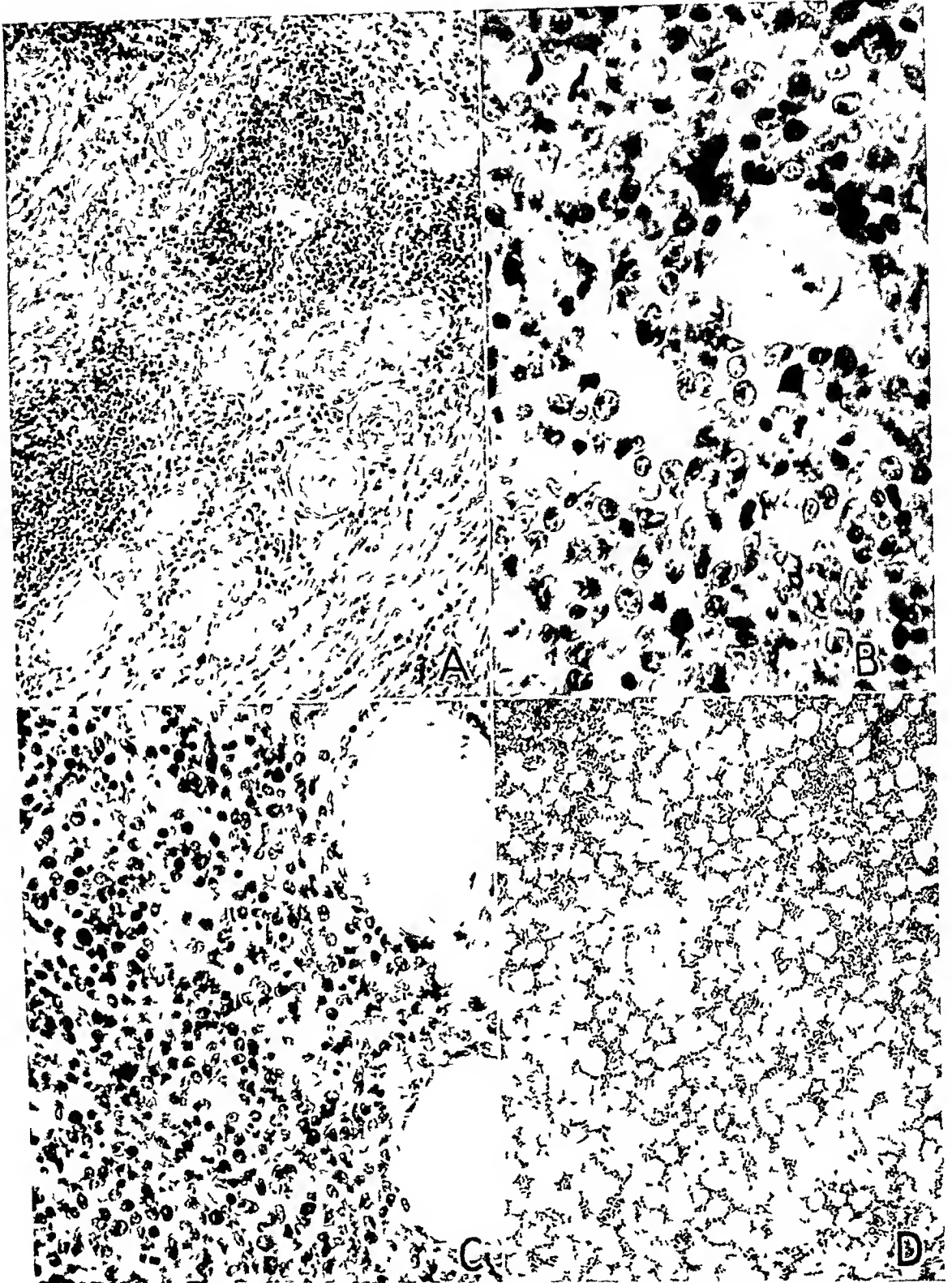


Fig 11—*A*, ganglion of a chicken with spontaneous neurolymphomatosis, the lymphoid infiltration does not appear to be neoplastic, $\times 120$ *B* ganglion of a chicken with transmitted lymphomatosis (strain 5), showing neoplastic infiltration, $\times 450$ *C*, ovarian lymphoma (strain 5), $\times 280$ *D*, the bone marrow of a chicken with spontaneous neurolymphomatosis showing no evidence of hyperplasia or lymphoid infiltration, $\times 60$. The sections were stained with eosin and azure II solutions. The magnifications given are approximate.

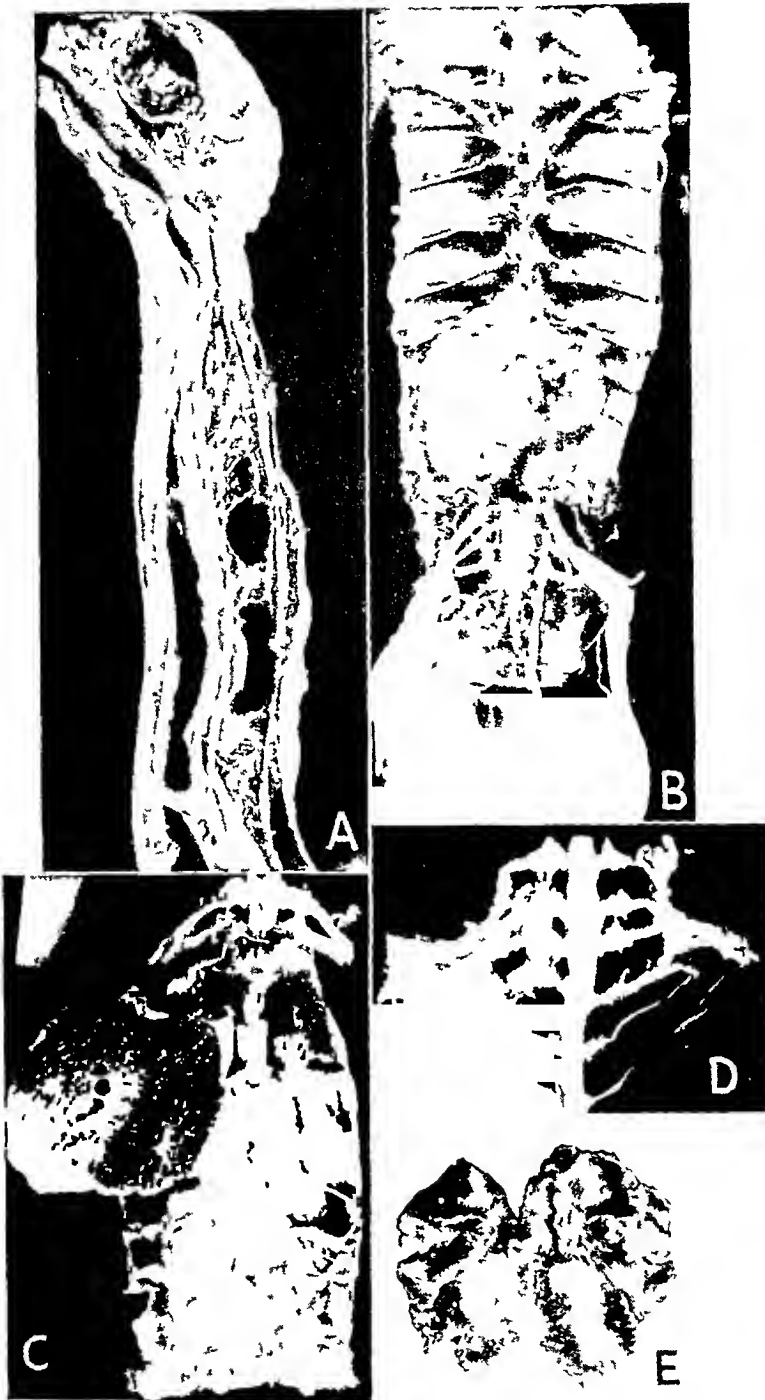


Fig 12—*A*, lymphomatosis of the vagus (strain 5) *B*, lymphoma infiltrating the adrenal, adjacent ganglions and upper lobe of the right kidney Lymphomatosis of the sciatic nerves and brachial plexuses *C*, lymphoma of the ovary infiltrating the upper lobes of both kidneys and adjacent parts of the right lung *D*, lymphomatosis of the brachial plexuses and ganglions *E*, lymphomatosis of the lung The last four pictures represent chickens with spontaneous neurolymphomatosis

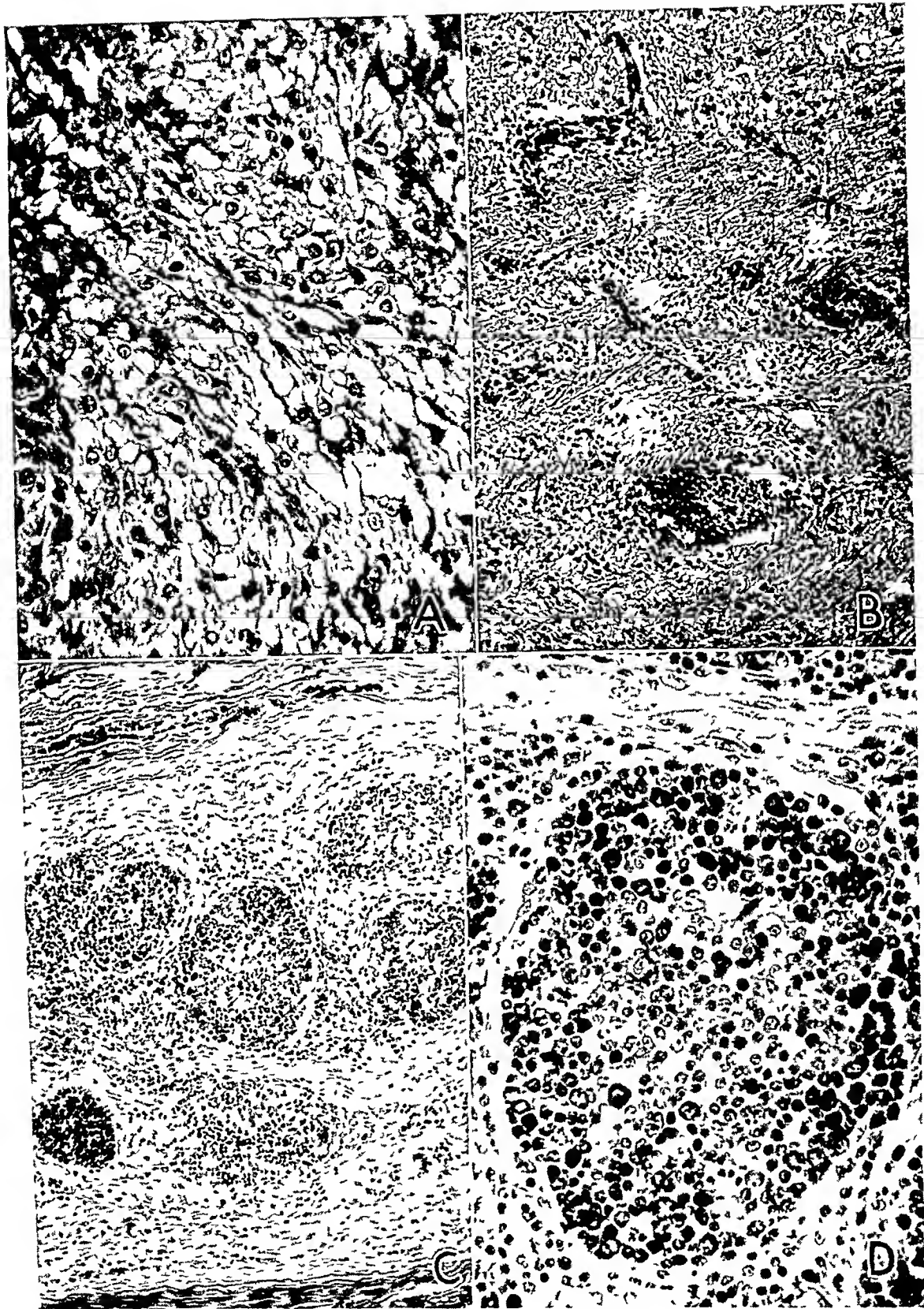


Fig 13—*A*, microscopic structure of a tumor of the brain found in a chicken that received an injection of tissues from paralyzed birds (strain 5), $\times 350$ *B*, perivascular small round cell infiltrations in the brain of a chicken that received an injection of strain 2, $\times 100$ *C* and *D*, lymphoid tissue with germinal centers in the sciatic nerve of a chicken at the site of intraneural injection of mouse brain carrying the virus of lymphogranuloma inguinale The sections were stained with eosin and azure II solutions The magnifications are approximately *C*, $\times 100$, *D*, $\times 300$

The spinal and sympathetic ganglions are favorite sites of infiltrations that are continuous with those of the peripheral nerves

Perivascular round cell infiltration in the brain and cerebellum (fig 13 *B*) was seen in numerous birds, but it is doubtful whether this infiltration was caused by the agent of neurolymphomatosis, for the following reasons (a) The infiltration in the brain was composed of small lymphocytes and occasional cells like monocytes, and mitosis among these cells was scant even in chickens in which the coexistent infiltration of peripheral nerves and viscera was composed of lymphocytes of medium size with abundant mitotic figures, (b) similar alterations in the brain are very often found in apparently normal chickens (Pappenheimer, Dunn and Seidlin), (c) these alterations have the characteristics of encephalitis, not those of a neoplasm

The cord was often normal even in the presence of extensive infiltration of the adjacent spinal ganglions. Less often it showed mild, occasionally extensive infiltration, which was usually perivascular and continuous with the meningeal and neural infiltration. It is possible that in these cases the lymphoid cells reached the cord by way of the peripheral nerves

In one chicken (3862) a spherical tumor about 0.8 cm in diameter was found in the cerebrum. The microscopic appearance of this tumor, shown in figure 13 *A*, resembled that of a spongioblastoma²²

Infiltration amounting to tumor formation about the inoculated sciatic nerve is shown in figure 10 *A*. The diagnosis of incipient neurolymphomatosis of the inoculated nerves offered great difficulties because of the nonspecific changes caused by the traumatism. The formation of lymphoid follicles about the nerve as shown in figure 13 *C* and *D* was considered a nonspecific alteration

Among the viscera, the ovary was the most frequent site of infiltration (figs 11 *C* and 12 *C*). It was enlarged to several times its normal size, its normal lobulated structure was obliterated by the yellowish-gray, soft, infiltrating lymphomatous tissue. In two instances, however, neurolymphomatosis was found in apparently healthy egg-laying chickens. The behavior of the blood-forming organs, bone marrow (fig 11 *D*), spleen and liver (fig 5 *C*) is noteworthy. In the majority of cases they showed no conspicuous alteration. The lymphomatous infiltration occasionally found in the bone marrow was nodular and replaced only a small part of the marrow, in the liver (fig 5 *C*) the infiltrations were periportal, in the spleen they were found both in the pulp and in the follicles. The normal structure of these organs was, however, invariably retained. Lymphomatous infiltration in the kidney

²² Dr Lewis D. Stevenson examined sections of the central nervous system and interpreted the lesions found

and adenal are shown in figure 12 *B* and *C*. The right lung in figure 12 *C* is normal, whereas that of figure 12 *E* is infiltrated by malignant lymphocytes.

TRANSMISSION WITH STRAIN 5

Origin of Strain—Twenty-three chickens with clinical symptoms of neurolymphomatosis, found in the state of Delaware, were killed after a period of observation of from one to thirteen days. Postmortem examination of thirteen of these chickens showed thickening of nerves with no tumor formation, another eight showed thickening of nerves and gross visceral infiltration or tumors, and in two there was no gross or microscopic evidence of neurolymphomatosis.

It is evident that neurolymphomatosis can be diagnosed fairly accurately by clinical examination since only two of the twenty-three chickens received failed to show gross evidence of the disease. These two chickens have not been studied microscopically. Most of these chickens, including the two that showed no gross evidence of neurolymphomatosis, were infested with tapeworms.

Unsuccessful inoculations were made in twelve chickens, each receiving one or more injections of blood or nerve emulsion from one or more chickens with spontaneous paralysis.

The first successful inoculations were made from a chicken (3878) with spontaneous neurolymphomatosis and the transmissible strain derived from this fowl is called strain 5.

Spontaneous neurolymphomatosis in this chicken was associated with lymphomatosis of the viscera and lymphatic leukemia. The association of lymphatic leukemia with neurolymphomatosis was hitherto unknown.

The bird was a much emaciated pale young Barred Rock hen that weighed 600 Gm. Its erythrocyte count was 2,950,000, the leukocyte count, 145,000, 92 per cent of the leukocytes were lymphocytes, mostly of small size (fig 9 *B* and *C*). The hematocrit values of the blood were erythrocytes, 33.5, leukocytes, 4.5, plasma, 62. The relatively low hematocrit value for leukocytes was due to the small size of the lymphocytes.

Both the legs and the wings were weak or paralyzed, the right more so than the left. At autopsy the vagus nerves, the brachial plexuses and their branches, and the lumbosacral plexuses and their branches were greatly thickened. The left brachial plexus was estimated to have a diameter of from three to five times normal, and attached to one of its branches there was a gray tumor nodule measuring 0.7 cm across. About one third of the left lung was the site of gray lymphomatous infiltration. In the skin of the neck there was a lymphoma about 2.5 cm in diameter, and a smaller lymphoma was found in the skin of the left leg. Surrounding the left carotid artery there was a fusiform tumor 0.5 cm across. There were gray spots in the voluntary muscles, and microscopic examination of these areas showed extensive interstitial infiltration with lymphocytes mostly of medium size, many in mitosis. The bone marrow was normal and contained abundant fat. Grossly, the liver appeared normal, the spleen was slightly enlarged, and its follicles were prominent.

The lymphocytes that caused the extensive infiltration of nerves and viscera and invaded the blood stream were mainly of medium and small size, indistinguishable morphologically from normal lymphocytes. The alterations found in the tissues were indistinguishable from lymphomatous neoplasms of mammals.

Experiments—Six chickens weighing from 850 to 1,100 Gm were given intravenous injections of 4 cc of the blood of chicken 3878. The inoculations were successful with one exception (table 4). This chicken (3849) has since been twice reinoculated intravenously with tissues derived from paralyzed chickens, without ill effects.

The second subpassages were also made by intravenous injections of large amounts of blood (from 1 to 10 cc) and, with the exception

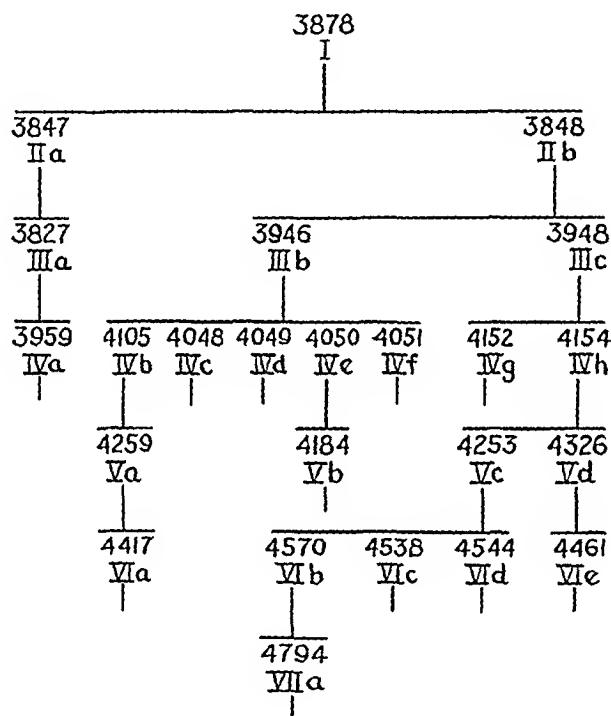


Fig 14—Passages of strain 5. The Roman figures show the number of the passage, the Arabic figures, the number of the chicken from which the passage was made. The results of inoculation are summarized in table 4.

of two chickens that died of early intercurrent infection of the upper respiratory passages, all inoculated chickens acquired neurolymphomatosis. The results of these and twenty subsequent passages are summarized in figure 14 and in table 4.

TRANSMISSION WITH STRAIN 6

Passages made with strain 6 neurolymphomatosis are shown in figure 15 and in table 5. The origin of this strain is somewhat obscure.

A chicken (3850) weighing 950 Gm received intravenously 21 cc of blood from two chickens with spontaneous neurolymphomatosis, and

TABLE 4—*Transmission of Neurolymphomatosis (Strain 5) with Cell-Containing Material*

Passage	Chickens Inocu- lated	Successful Inocula- tions	Material Injected	Route of Injection*	Length of Life and Results of Injection†
I	6	4	Blood	Intrav	K8+, K32+, D36+, D40—, D46+, alive —
IIa	4	2	Blood	Intrav	D34—, D51—, K59+, K78+
IIb	4	4	Blood	Intrav	K43+, K49+, K75+, K77+
IIIa	3	1	Blood	Intrav	D36—, D48—, K84+
IIIb	7	6	Blood	Intrav	K24+, K41+, K66+, K77+, K97+, + recovered, alive —
IIIc					
12/31	3	1	Blood	Intrav	D55—, K55+, K73—
1/ 7	4	4	Blood	Intrav	K36+, K51+, K56+, K71+
IVa					
11/23	4	1	Blood	Intrav	K64+, alive —, alive —, alive —
11/27	4	3	Tumor	Intram	D68+, D68+, K72+, alive —
IVb					
12/ 9	4	1	Blood	Intrav	D40—, K45+, D72+
12/18	4	4	Blood and spleen Tumor	Intrav Intram	{ D44+, K54+, K70+ K123+
IVc	3	2	Blood	Intrav	D48±, K48+, D66+
IVd	6	2	Blood	Intrav	K54+, K77—, D101+, alive —, alive —, alive —
IVe					
11/23	4	2	Blood	Intrav	K37—, K37—, K37+, K155+
11/29	3	2	Tumor	Intram	K66+, K105+, alive —
IVf	1	1	Blood	Intrav	K65+
IVg	3	0	Blood	Intrav	D51—, K103—, alive —
IVh	4	3	Blood	Intrav	D53+, K65+, K96+, K99—
Va	3	1	Blood	Intrav	K68+, D98—, alive —
Vb	3	1	Blood	Intrav	K59+, D90±, alive —
Vc	7	4	Blood	Intrav	K40—, K43+, K49+, D56+, D61±, K104+, K162—
	4	2	Blood	Intran	D33—, D34+, K38—, D51+
Vd	3	2	Blood	Intrav	K58+, K79+, K112—
	3	2	Blood	Intran	D29+, D48+, K112—
VIa	2	1	Tumor	Intran	D25+, K145—
VIb	3	2	Blood and tumor	Intran	K36+ K60+, K123—
VIc	2	2	Blood	Intran	D34+, D43+
VID	3	0	Blood	Intrav	K144—, K144—, K144—
VLe	3	1	Tumor	Intran	D39—, D60+, K145±
VIIa	3	0	Blood and nerve	Intran	D22±, K124—, K124—
Total	110	61			

* The abbreviation Intrav means intravenous, Intram, intramuscular, Intran, intraneural
† K signifies that the chicken was killed, D, that the chicken died. The numerals state the length of life. The plus and minus signs indicate that neurolymphomatosis was (+) or was not (—) found on examination, or that the success of the inoculation was doubtful (±). Thus K8+ means that a chicken killed thirty two days after inoculation showed evidence of neurolymphomatosis. "Alive—" means that the chicken was unaffected after a period of from four to five months. Most of the chickens designated in the tables as "alive" were used for studies of immunity in relation to fowl paralysis.

five days later it was inoculated with a suspension of cells of a lymphomatous tumor of a paralyzed chicken. The purpose of this experiment was to shorten the period of incubation and to overcome the resistance of the host by massive doses. Six days after the inoculation the chicken showed conspicuous weakness of the left leg and left wing, and two healthy chickens were each given an injection of 5 cc of its blood. Both these chickens died with neurolymphomatosis (table 5), but chicken 3850, on which autopsy was done three days after these inoculations were made, showed peritonitis and pneumonia with no gross or microscopic evidence of neurolymphomatosis. Nevertheless, it carried the agent of neurolymphomatosis.

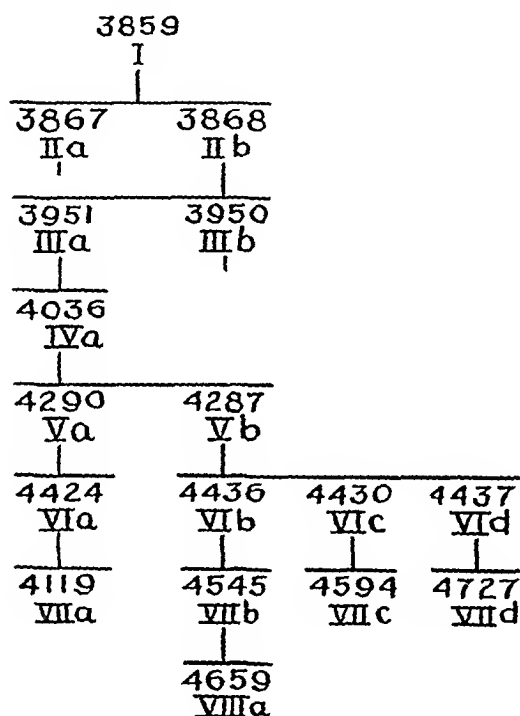


Fig 15—Passages of strain 6. The Roman figures show the number of the passage, the Arabic figures, the number of the chicken from which the passage was made. The results are summarized in table 5.

Three of the four chickens of the first subpassage (IIa of table 5) were lost in an epidemic of fowl pox and were discarded without microscopic study. One chicken of the second passage (IIb) presented paralysis, but four chickens (passage IIIb) inoculated with its blood were unaffected, another chicken of the second passage showed leukemic myelocytomatosis with no evidence of neurolymphomatosis. Since this was the only instance of leukosis other than lymphomatosis observed in this study involving approximately three hundred chickens, no significance can be attached to its occurrence. This chicken was probably a carrier of the agent of neurolymphomatosis, for one of the three

chickens inoculated with its blood (4036, passage IIIa) acquired typical neurolymphomatosis. Chicken 4036 is one of the three chickens designated in figure 15 and in tables 4 and 5 as successfully inoculated because its blood contained the transmitting agent in spite of the absence of the anatomic changes of neurolymphomatosis.

TABLE 5—*Transmission of Neurolymphomatosis (Strain 6) with Cell-Containing Material*

Passage	Chickens Inoculated	Successful Inoculations	Material Injected	Route of Injection*	Length of Life and Results of Injection*
I	2	2	Blood	Intrav	K49+, K59+
IIa	4	0	Blood	Intrav	K20—, K25—, K25—, alive —
IIb	4	2	Blood	Intrav	K23+, K39+, alive —, alive —
IIIa	3	1	Blood	Intrav	K76+, K77—, K77—
IIIb	4	0	Blood	Intrav	Alive —, alive —, alive —, alive —
IVa					
12/16	3	0	Blood	Intrav	D25—, D30—, alive —
12/19	6	4	Blood or nerve	Intrav or intran	K38+, K53+, D44+, K56+, K78—, alive —
Va	3	3	Blood or nerve	Intran	K33+, K56+, K65+
Vb	9	8	Blood or nerve	Intrav or intran	K40+, D47+, D53+, K54+, K62+, D95—, D97+, K111+, K117+
VIa	3	1	Nerve	Intran	K32+, K48±, K154—
VIb	5	3	Blood	Intrav or intran	K41+, K50+, D55+, K66—, K114—
VIc					
VIe	2	2	Nerve	Intran	D44+, D62+
VIId	4	3	Blood	Intrav	D49+, K56+, K82+, K156—
VIIa	3	1	Blood or nerve	Intran	K88+, K102—, K116—
VIIb	3	3	Blood or nerve	Intran and intran	K36+, K51+, K122+
VIIc					
VIId	3	0	Blood	Intrav and intran	K124—, K124—, K124—
VIIId	3	1	Blood or tumor	Intran	D20—, D24+, K120—
VIIJa	2	1	Blood	Intran	K55+, D96—
Totals	66	35			

* The abbreviations are explained in the footnote to table 4

The history of further subpassages is shown in table 5. The introduction of intraneural injection has greatly increased the percentage of successful inoculations, and the extensive infiltration in and about the nerve receiving the injection has revealed the success of the injection shortly after inoculation.

PRESENCE OF THE TRANSMITTING AGENT IN THE BLOOD

The first question that confronted us was whether transfers with blood should be made at the early or at the late stage of the disease. It is known that in most common virus diseases the appearance of

antibodies is followed by disappearance of the virus from the blood, but leukosis is readily transmitted with blood at an advanced stage of the disease. Quantitative studies on the concentration of the transmitting agent of leukosis and of Rous sarcoma in the blood have thus far, to our knowledge, not been made, but our experience with leukosis indicates that the disease is transmissible with the blood of leukotic chickens at all stages of illness. The results of an experiment made with strain 5 are recorded in table 6. The chicken whose blood was used in this experiment was inoculated on September 1 and showed the first signs of paralysis on October 13. Tests for the presence of the transmitting agent in its blood were made on October 13, October 31 and November 13. Two days later, when moribund, the chicken was killed. It showed extensive thickening of numerous nerves but no infiltration or tumors in the viscera.

TABLE 6—*Data on Transfers of Strain 5 by Injections of Blood at Successive Stages of Illness*

Date of Injection	Result with 0.5 Cc	Result with 5 Cc
October 13	K24+	K41+, alive —
October 13 and 31	+ recovered	K77+
October 31	K66+	K97+
November 13	D36+, D54+	

Similar experiments were made with the blood of a chicken that was inoculated on September 1 and showed the first signs of paralysis on October 31. The first inoculation, made on October 31, was much less successful than the second, made with blood taken from the heart immediately after death (November 17). Inoculations on October 31 with 0.5, 2 and 10 cc. of blood gave, respectively, negative results in a chicken that died at 55 days, neurolymphomatosis in one that was killed at 55 days and negative results in one that was killed at 73 days. Inoculations on November 17 with 0.2 cc. of blood gave neurolymphomatosis in chickens killed at thirty-six, fifty-one, fifty-six and seventy-one days, respectively, after inoculation.

These experiments show that the transmitting agent circulates in the blood of the paralyzed chicken during the entire period of manifest disease. Economic reasons prevented us from determining accurately the concentration of the transmitting agent in the blood. In subsequent work, however, the transfers were successfully made, as with leukosis, from chickens with advanced disease. Nevertheless, quantitative comparative determination of the presence of oncogenic agents and of common viruses in the blood at various stages of illness is desirable.

Concentration of the Transmitting Agent in the Blood—Intraneural inoculations were made with approximately from 0.01 to 0.02 cc. of

blood, and since the majority of the chickens thus inoculated presented the disease within a comparatively short period of time, it is probable that the minimal infecting dose is much smaller.

Before the discovery of the efficiency of the intraneural route, attempts were made to determine the amount of blood required to transmit paralysis by the intravenous route. The results of such an experiment were as follows. With 1 cc, two chickens killed at forty-nine and one hundred and four days after inoculation showed neurolymphomatosis. With 0.01 cc, one chicken that died at fifty-six days presented neurolymphomatosis, while in another that died at sixty-one days the success of the inoculation was doubtful. With 0.0001 cc, one chicken that was killed at forty-three days showed neurolymphomatosis, while two that died at forty and one hundred and sixty-two days did not show any evidence of the disease.

TABLE 7—*Experimental Study of Concentration of Transmitting Agent in Blood*

Experiment	Amount of Blood Injected, Cc	Results of Injection*	Experiment	Amount of Blood Injected, Cc	Results of Injection*
1	1	K75+	4	0.02	D101+, alive —
	2	K43+		0.2	K54+, K166—
	3	K49+		2	K77—, alive —
	10	D77+			
2	0.5	+ recovered, K24+, K66+	5	0.5	Alive —, alive — K64+, alive —
	5	K77+, K41+, K97+, alive —	6	0.5	K37+ K155+
3	0.5	D55—	7	0.02	D48+
	2	K57+		0.2	D66+
	10	K73—		2	K48+

* The abbreviations are explained in the footnote to table 4.

Preceding this experiment from 0.02 to 10 cc of blood was used for intravenous inoculations, but the results appeared to be independent of the amount of blood injected, as shown in table 7.

INTRANEURAL TRANSMISSION

Failure of previous workers to increase the percentage of successful inoculations by intraneural injections or to cause local infiltration of the nerves receiving injections at first discouraged attempts to transmit the disease by this route, but when it was recognized that neurolymphomatosis is allied to Rous sarcoma and Ellermann's leukosis, these failures seemed paradoxical. For if the lymphocyte is the neoplastic cell in neurolymphomatosis and the peripheral nerves and ganglions are the common sites of infiltration, intraneural introduction of the malignant cells should bring about infiltration of the nerves receiving injections. Comparative intravenous and intraneural inoculations with the free virus, on the other hand, might determine whether the virus has a

special affinity for the nerves, which are secondarily invaded by lymphocytes, or for lymphocytes, which it renders malignant and endows with special affinity for the nerves

The experiments recorded in table 8 show that with the blood of paralyzed chickens intraneural inoculations are more often successful than intravenous inoculations

TABLE 8—*Comparison of Intraneural with Intravenous Transfer*

Route of Injection		Results of Inoculation	
Intravenous		K58+, K79+, K112—	
Intraneural		D29+, D18+, alive —	
Intravenous		D56+, D61—	
Intraneural		D33—, D38—, D51+, D34+	
Intravenous		K66—, K114—	
Intraneural		K41+, K50+, D55+	
	Totals	Chickens Inoculated	Chickens with Neurolymphomatosis
Intravenous		7	3
Intraneural		10	7

TABLE 9—*Comparison of Emulsion of Infiltrated Nerves with Whole Blood as a Medium of Transfer*

Material Injected	Amount Injected, Cc	Route of Injection	Results of Injection
Blood	0.5	Intrav	K54+, D95—, K97+
Nerve*	0.01	Intrav	K40+, K110+, K117+
Nerve*	0.01	Intran	D47+, D53+, K62+
Blood	1	Intrav	K78—, K115—
Nerve*	0.01	Intran	K38+, K63+
Nerve*	0.01	Intran and intrav	D44+, K56+
Blood	0.1		
	Totals	Chickens Inoculated	Chickens with Neurolymphomatosis
Intravenous		8	5
Intraneural		7	7

* Suspension of infiltrated nerves

An emulsion of infiltrated nerves transmits the disease as well as, if not better than, whole blood (table 9)

In the last two chickens referred to in table 9 an emulsion of infiltrated nerves was injected into one sciatic nerve and blood into the other. The nerve inoculated with emulsion of nerve cells was more thickened than that inoculated with blood. Nerve tissue itself is not required for transmission of the disease, for emulsions of lymphomatous tumors of the ovary and skin as well as blood readily transmit the disease.

In a later experiment the blood of a paralyzed chicken was injected into the right sciatic nerve and the blood of a normal chicken into the left sciatic nerve in two chickens. One remained healthy, the other, when killed fifty-five days after these injections, showed extensive thickening of both sciatic nerves associated with infiltration of several other nerves. This observation raised doubt concerning the feasibility of testing different materials by multiple intraneural injections into one chicken. Numerous control intraneural inoculations have shown that neurolymphomatosis can be produced only with tissues of lymphomatous chickens (see p. 38). It is possible that in birds carrying the virus of neurolymphomatosis localization of lymphomatous infiltration is facilitated by trauma. Such is the case with the virus of yellow fever.²³

The following observations suggest that the blood stream is the major channel through which the agent spreads from one nerve to distant nerves.

(a) The inoculation into the sciatic nerve was made midway between the sciatic foramen and the popliteal fossa. The infiltration was extensive distal from the site of inoculation but was slight proximal to it.

(b) The spinal cord in neurolymphomatosis shows slight or no infiltration. There is only slight interstitial or perivascular infiltration in the cord even in instances in which the spinal ganglions are so extensively infiltrated that their structure is barely recognizable.

(c) The distant lesions after intraneural injection are as varied as after intravenous injection, and nerve tissue between the diseased and the inoculated nerve often appears normal.

(d) The blood contains the transmitting agent in high concentration and reproduces a disease indistinguishable from spontaneous neurolymphomatosis.

These observations are in accord with those summarized by Abel,²⁴ according to which the bulk of the material injected into and about nerves does not follow nerve routes but passes into the subclavian vein by way of the endoneural and perineural lymphatics.

The vasa lymphacea ischiadica of chickens accompany the sciatic nerve in the distal two thirds of the thigh (Baum²⁵) and, passing along the median side of the os femoris, enter the peritoneal cavity and end in the thoracic duct.

Here the objection may be raised that both lymphocytes and virus might travel along the nerve fibers without causing anatomic alterations.

23 Sawyer, W. A., and Lloyd, W. J. *Exper. Med.* **54** 533, 1931.

24 Abel, J. J. *Science* **79** 121, 1934.

25 Baum, H. *Ztschr. f. Anat. u. Entwicklungsgesch.* **93** 1, 1930.

Ease of transmission with cell-containing material and difficulty of transmission with injured cells suggest that the major factors in the spread of the disease are lymphocytes. The ease with which the disease is transmitted by blood suggests that the lymphocytes reach distant nerves by way of the blood stream. That infiltration extends along the inoculated nerve is certain, for the sciatic nerve becomes uniformly thickened distally from the site of injection, proximally, too, the infiltrations may extend as far as the cord. In one instance, in which the ulnar nerve was inoculated, it was uniformly thickened throughout its course. Thus some lymphocytes introduced in peripheral nerves may reach the meninges, as do brominized oil,²⁶ prussian blue²⁷ and some neurotropic viruses,²⁸ but systemic neurolymphomatosis appears to be the result of intravenous dissemination of the causative agent.

CONTROL INTRANEURAL INJECTIONS

The specificity of the alterations produced by intraneural injections of lymphomatosis strains 2, 5 and 6 was tested as follows.

Leukosis strain 1 produces erythroleukosis and myeloblastic leukemia. Its characteristics remained unchanged during five years of observation. The results of injections made with the blood of chickens with myeloid leukemia associated with erythroleukosis were as follows.

Experiment 1—Intravenous injections gave myeloid leukemia in one chicken that died at thirty-nine days, and erythroleukosis in four chickens, three of which died at forty, forty-seven and fifty-one days, respectively, and one of which recovered. Intraneural injections gave erythroleukosis in two chickens that died at sixty-two and seventy days, myeloid leukemia and erythroleukosis in one that died at sixty-nine days, myeloid leukemia in one that died at seventy-four days, and no evidence of disease in one that was killed at ninety-one days.

Experiment 2—Intravenous injections yielded negative results in two chickens killed at twenty-five and one hundred and three days and erythroleukosis in one killed at thirty-five days. Intraneural injections yielded erythroleukosis in a chicken that died at thirty-nine days and negative results in two chickens killed at one hundred and three days.

There was only a slight thickening of the nerves, similar to that caused by normal blood, at the site of puncture with the needle.

These experiments show that leukosis strain 1 is readily transmitted by intraneural inoculation, but it does not produce neurolymphomatosis.

26 Sullivan, W. E., and Mortensen, O. A. *Anat Rec* **59** 493, 1934.

27 Clark, W. E. LeG. Report to Committee on Vaccination on an Anatomical Investigation into Routes by Which Infection May Pass from Nasal Cavities into Brain. Reports on Public Health and Medical Subject, no. 54, Ministry of Health, London, His Majesty's Stationery Office, 1929.

28 Hurst, E. W. *J Path & Bact* **33** 1133, 1930, *J Exper Med* **59** 729, 1934.

Eight chickens given injections of the blood of normal chickens remained healthy

In association with Dr A Grace, I inoculated three chickens intraneurally with the brain tissue of a mouse carrying the neurotropic virus of lymphogranuloma inguinale²⁹ These chickens, killed twenty, fifty and seventy days after inoculation, showed at the site of inoculation formation of lymphoid tissue with germinal centers (fig 13 C and D) Aside from this there was no lesion suggestive of lymphomatosis One chicken inoculated intraneurally with a cell suspension of transmissible lymphomatosis of mice gave no evidence of neurolymphomatosis

Summary—Intraneural inoculations are followed by neurolymphomatosis only when the material injected is obtained from chickens with lymphomatosis

RELATION OF LYMPHOMATOSIS OF VISCERA TO LYMPHOMATOSIS OF NERVES

The association of neurolymphomatosis with lymphomatous infiltration or lymphomas of the viscera has been noted by most of those who have worked with this disease, and observations made by Pappenheimer, Dunn and Cone⁵ and several other workers suggest that both are caused by the same agent This view has been contradicted by Mathews,^{8b} who found that lymphomatosis is often unassociated with infiltration of the nerves This is true for a common variety of lymphomatosis that is characterized by great enlargement of the liver ("big liver" disease, hepatolymphomatosis)

Experiments were made to determine the relationship of lymphomatous tumors occurring among the passages of strain 5 to the infiltration of the nervous system In experiment 1 a suspension of infiltrated nerves produced extensive lymphomatous infiltration of the inoculated nerve, but it did not produce infiltration or tumors in the inoculated breast muscle Experiments 2, 3 and 4 showed that the tissue of lymphomatous tumors occurring among the passages of strains 5 and 6, unlike strain 2 or lymphomas of mice, does not produce infiltration in the inoculated breast muscle, but may produce neurolymphomatosis unassociated with infiltration of the viscera

Experiment 1—An emulsion of infiltrated nerves (strain 5) was injected into both the right pectoral muscle and the right sciatic nerve in three chickens, and blood was injected into the left pectoral muscle and the left sciatic nerve in the same chickens The birds were killed thirty-six, fifty-one and one hundred and twenty-two days after the injections were made The sites of the intramuscular injections could not be detected, but all nerves that received injections were much thickened, those inoculated with nerve emulsion to a greater extent than those inoculated with blood

²⁹ Grace, A Proc Soc Exper Biol & Med **32** 71, 1934

Experiment 2—Small pieces from a lymphomatous tumor (strain 5), about 8 by 6 by 2 cm, which had infiltrated the skin and muscles of the wing of a paralyzed chicken, were injected into the muscles and subcutaneous tissue of the breast and leg in four chickens. One of these chickens remained healthy, the second became paralyzed and died sixty-eight days after inoculation with gross evidence of extensive neurolymphomatosis unassociated with lymphomatosis of other organs. The third, killed sixty-eight days after injection because of fowl pox, showed on microscopic examination conspicuous lymphoid infiltrations of nerves and of the sympathetic ganglion about the adrenal. The fourth, killed seventy-two days after injection because of roup, showed on microscopic examination distinct lymphoid infiltration of several nerves. None of these chickens showed infiltration at the sites of the injections.

Experiment 3—Lymphomatous tumor tissue from a paralyzed chicken (strain 5) was injected into the pectoral muscles of three chickens. None of these chickens had infiltration in the inoculated muscles. One remained healthy, the second presented characteristic paralysis with no tumors, and the third showed diffuse lymphomatosis.

Experiment 4—In this experiment four chickens were inoculated by way of the pectoral muscle with tumor tissue of a paralyzed chicken (strain 5). All four acquired neurolymphomatosis unassociated with lymphomatous infiltration of the inoculated muscle or of the viscera. Since each of these chickens also received an intravenous injection of blood, the general disease may have been produced with blood.

In the following experiments lymphomatous tumor tissue occurring in association with neurolymphomatosis was injected into the sciatic nerves of healthy chickens, and it produced typical neurolymphomatosis unassociated with lymphomatosis of the viscera.

Experiment 5—Tissue of an ovarian lymphoma occurring in a paralyzed chicken (strain 5) was injected into the sciatic nerve in three chicks. One that died thirty-nine days after inoculation and another that died sixty days after inoculation showed extensive lymphomatous infiltration of the inoculated nerves, the third remained healthy.

Experiment 6—Tissue of a lymphoma of the breast muscle of a paralyzed chicken was injected into the sciatic nerve in two chickens. One of these died thirty-five days after inoculation with extensive lymphomatosis of the inoculated nerve and general neurolymphomatosis unassociated with tumor formation. The second chicken remained healthy.

Experiment 7—Tissue of an ovarian lymphoma of a paralyzed chicken (strain 5) was injected into the left sciatic nerve and blood of the same paralyzed chicken was injected into the right sciatic nerve in three chicks. Two died from twenty to twenty-four days after inoculation, showing neurolymphomatosis, mainly of the inoculated nerves, the third remained healthy.

These experiments suggest that tumor tissue and suspensions of infiltrated nerves transmit neurolymphomatosis more readily than blood, and support the view that the lymphocytes that form tumors and infiltrate nerves are the cells responsible for the transmission of the disease. It may be assumed that transmission of the disease is due to a virus and that the lymphocytes are carriers of this virus, but the

experiment reviewed in the next section failed to demonstrate transmission by cell-free material

The tumor tissue in all these experiments was derived from paralyzed chickens. Lymphomatosis of the viscera unassociated with lymphomatosis of the nervous system was found only on gross examination. In the following experiment, blood and tumor of a chicken that had extensive lymphomatous infiltration of the viscera, but only microscopic neural infiltrations, produced extensive neurolymphomatosis.

Experiment 8—Chicken 4570 (strain 5) died ninety-two days after intraneural inoculation with blood frozen at -30°C for thirty minutes. At autopsy, numerous lymphomas were found in the heart, lung and skin, the largest being about 1 cm in the longest diameter. Microscopic examination showed moderate lymphomatosis of the peripheral nerves. The heart blood of this chicken was injected into the left sciatic nerve and tumor tissue into the right sciatic nerve in three chickens. Two of these, killed thirty-six and sixty days after inoculation, showed neurolymphomatosis with extensive infiltration of both inoculated nerves, the third remained healthy.

Summary—Tissue from lymphomatous tumors occurring among passages of strains 5 and 6, when inoculated intraneurally or intramuscularly into healthy chickens, produced neurolymphomatosis with or without lymphomatosis of viscera. After intramuscular injection it failed to produce tumors in the inoculated muscles, nevertheless, it produced neurolymphomatosis. After intraneural injection it produced extensive lymphomatous infiltration of the inoculated nerves.

ATTEMPTS TO DEMONSTRATE CELL-FREE TRANSMISSION

Effect of Freezing and Thawing—Freezing inactivated the transmitting agent in all but one experiment (the fourth of table 10).

Previous experiments have shown that blood cells are destroyed when exposed for thirty minutes to a temperature below approximately -15°C ,³⁰ but the microorganisms tested and the agents of chicken leukosis and sarcoma³¹ are resistant to much lower temperatures. In the first six experiments of table 10 the material to be frozen was sealed in a test tube and submerged for thirty minutes in alcohol cooled with solid carbon dioxide to a temperature of from -25 to -30°C . In the last experiments an attempt was made to determine the subzero temperature at which the transmitting agent became inactivated. From 0.2 to 1 cc of blood was injected intravenously and about 0.02 cc intraneurally.

The meaning of these experiments is obscure. It is improbable that a virus can be destroyed by thirty minutes' exposure to a temperature of from 20 to 30°C . The possibility remains that the malignant lymphocytes contain a filtrable agent which is inactive in the absence of live cells, requiring their presence to obtain a foothold in a new host.

³⁰ Furth, J., Seibold, H. R., and Rathbone, R. R. *Am J Cancer* **19** 521, 1933. Furth, J. *J Exper Med* **61** 423, 1935.

³¹ (a) Stubbs, E. L., and Furth, J. J. *J Exper Med* **61** 593, 1935. (b) Furth ^{4a}

These experiments demonstrate the necessity of using live cells for transmission of the disease, and explain the unsuccessful attempts of those who have attempted transmission by crushing the cells with the purpose of obtaining the hypothetical virus

TABLE 10—*Effect of Freezing on the Transmitting Agent*

Material Injected	Route of Injection*	Results of Injection*			
Fresh blood	Intrav	D36+, D54+			
Frozen blood	Intrav	Alive —, alive —			
Fresh blood	Intrav	K68+, K99—, alive —			
Frozen blood	Intrav	D90—, K109—, K160—			
Fresh blood	Intrav	K49+, K104+			
Frozen blood	Intrav	D53—, K101—			
Fresh blood	Intran	D56+, D61±			
Frozen blood	Intran	K58+, D64—, D78—, D92+			
Fresh blood	Intran	K33+, K65+, D56+			
Frozen blood	Intran	K101—, K150—, K150—			
Fresh nerve	Intran	D16+, D44+, D62+			
Frozen nerve	Intran	D35—, D59—, K67—			
Fresh blood	Intran	D43+, D47+			
Blood frozen at —10 and —15 C	Intran	D19—, D89—, D90—			
Blood frozen at —20 C	Intran	K28—, D80—, K122—, K122—			
Totals	Chickens Inoculated	Chickens with Neurolymphomatosis	Chickens in Which Result Was Doubtful	Chickens Unaffected	
Fresh material	17	14	1	2	
Frozen material	24	2	0	22	

* The abbreviations are explained in the footnote to table 4

TABLE 11—*Results of Inoculations with Plasma*

Material Injected	Amount Injected, Cc	Route of Injection*	Results of Injection*
Whole blood	0.5	Intrav	D36+, D54+
Plasma	0.5	Intrav	K130±, K202—
Blood cells	0.5	Intrav	K129+, K202—
Whole blood	0.2	Intrav	K96+, K99—
Plasma	0.2	Intrav	K78—, K117+
Blood cells	0.2	Intrav	D53+, K65+
Whole blood	0.02	Intrav	K66—, K114—
Plasma	0.02	Intrav	D30—, D62—, D66—
Plasma	1.0	Intrav	K80—, K163—
Whole blood	0.02	Intran	K41+, K50+, D55+
Plasma	0.02	Intran	K34—, D84—, K113— (sarcoma)

* The abbreviations are explained in the footnote to table 4

Attempts at Transmission with Plasma—One of nine chickens inoculated intravenously with plasma presented paralysis, all of three chickens inoculated intraneurally remained healthy (table 11)

The plasma was obtained by spinning heparinized blood at approximately 1,000 revolutions per minute and recentrifugating the plasma at approximately 2,000 revolutions per minute for from twenty to thirty minutes. The plasma was carefully removed to avoid its contamination with cells. Previous experiments had shown that plasma obtained under these conditions was cell-free.

Attempts to transmit lymphomatosis and myelosis of mice with plasma similarly obtained had been uniformly unsuccessful.³⁰ Failure of intraneural inoculations with cell-free material other than plasma made in a large number of chicks will be described in the next section. The sarcoma that appeared in the lumbar region in one of the chickens inoculated with plasma proved to be transmissible by tumor filtrates and desiccates, but its virus did not produce paralysis (strain 15).

TABLE 12—*Effect of Drying on the Transmitting Agent*

Material Injected	Route of Injection*	Age of Dried Material, Days	Results of Inoculation*		
Fresh blood	Intrav	—	D31+, D54+, K129+, K200—		
Dried blood	Intrav	86	K120—, K163±, K163—, K163—		
Fresh blood	Intrav	—	K25+, K43+, K49+, D77+		
Dried blood	Intrav	159	K36—, D40—, K163—		
Fresh blood	Intrav	—	D44+, K54+, K70+, K123+		
Dried blood	Intrav	61	K99+, K163—, K163—		
Mixture of 3 dried samples	Intrav	61 to 150	D54—, K163—, K163—		
Fresh nerve	Intran	—	D16±, D44+, D62+		
Dried nerve and blood	Intran and intrav	1	D33—, D41—, D43—, D45—, D101—, K148—		
Dried blood	Intran	1	D45—, K148—, K148—		
Totals			Experiments	Chickens Inoculated	Successful Injections
Fresh (control) material			4	15	13
Dried material			6	22	1

* The abbreviations are explained in the footnote to table 4.

Attempts at Transmission with Desiccated Tissues—Table 12 is a summary of unsuccessful attempts to preserve by drying the transmitting agents of strains 5 and 6. Only one of twenty-two chickens given injections of dried tissues of paralyzed birds died with paralysis.

Rous and Murphy³² found that some agents of sarcoma can be preserved by drying, whereas others cannot. Drying was usually successful with our leukosis strain 1,³³ sarcoma strains 11 and 15³⁴ and sarcoma-leukosis strain 13 (Stubbs and Furth^{31a}), often successful with leukosis strain 2³⁵ and occasionally successful with osteochondrosarcoma-leukosis strain 12³⁴.

³² Rous, P., and Murphy, James. J. Exper. Med. **19** 52, 1914.

³³ Furth, J. J. Exper. Med. **55** 495, 1932.

³⁴ Furth, J. Unpublished Work.

³⁵ Furth ^{4a, 34}

The technic of drying from the frozen state has been described^{1a} The dried material was stored in the icebox in sealed test tubes, and only after the corresponding fresh samples were found to be highly active were the stored samples used for the injections Each chicken received approximately 50 mg of dried blood taken up in Locke's solution In the last two experiments dried blood was injected twenty-four hours after the injection of the fresh blood In the first four experiments dried blood was injected intravenously, in the fifth experiment dried blood was injected intravenously and into one sciatic nerve, while the other sciatic nerve received an injection of dried suspension of an infiltrated nerve In the sixth experiment dried blood was injected into one sciatic nerve and a suspension of infiltrated nerve was injected into the other sciatic nerve The uniform results obtained after drying under identical conditions each of our different oncogenic strains makes it unlikely that failure to preserve the agents of neurolymphomatosis by drying is due to faulty technic

Summary—Neurolymphomatosis occurred in four of the fifty-eight chickens inoculated with material free from viable cells The number of positive results is too small to be significant If neurolymphomatosis is caused by a virus, the conditions under which this virus acts remain to be demonstrated

TABLE 13—*Summary of Attempts to Demonstrate Transmission of Neurolymphomatosis by Cell-Free Material*

Material Injected	Chickens Inoculated	Chickens with Neurolymphomatosis
Dried	24	1
Frozen	10	2
Plasma	24	1
Total	58	4 (7%)

III CONTROL OBSERVATIONS REGARDING TRANSMISSIBILITY OF LYMPHOMATOSIS

The frequent occurrence of spontaneous neurolymphomatosis suggested a study of its incidence among our laboratory animals

UNINOCULATED CHICKENS

Uninoculated chickens that could be regarded as controls were few in these studies, mainly because we assumed that each strain was well controlled by the rest of our flock The fate of twenty-nine uninoculated chickens observed mainly during the first phase of these studies is, however, of some interest The vertical line of figure 16 shows the number of chickens, the horizontal line the period during which they were observed in the laboratory, for example, after three months twenty-five of the twenty-nine chickens were alive and none of the four that had died had leukosis or sarcoma Later three of the twenty-nine uninoculated control chickens died with aleukemic lymphomatosis, but none of them showed gross evidence of neurolymphomatosis, one

died with sarcoma. These as well as the similar figures given in previous reports urge caution in interpreting the results of transmission experiments made without controls and on small numbers of animals. Although paralysis was not seen in this series, one of forty young chickens ("broilers") received from the farmer who supplied us with most of our birds showed advanced paralysis four days after arrival.

The incidence of spontaneous neoplasms among these twenty-nine control chickens was unexpectedly high. The chickens used in studies of leukosis strains 1 and 2 were usually destroyed three months after inoculation. Only one instance of leukosis occurred among the twenty-

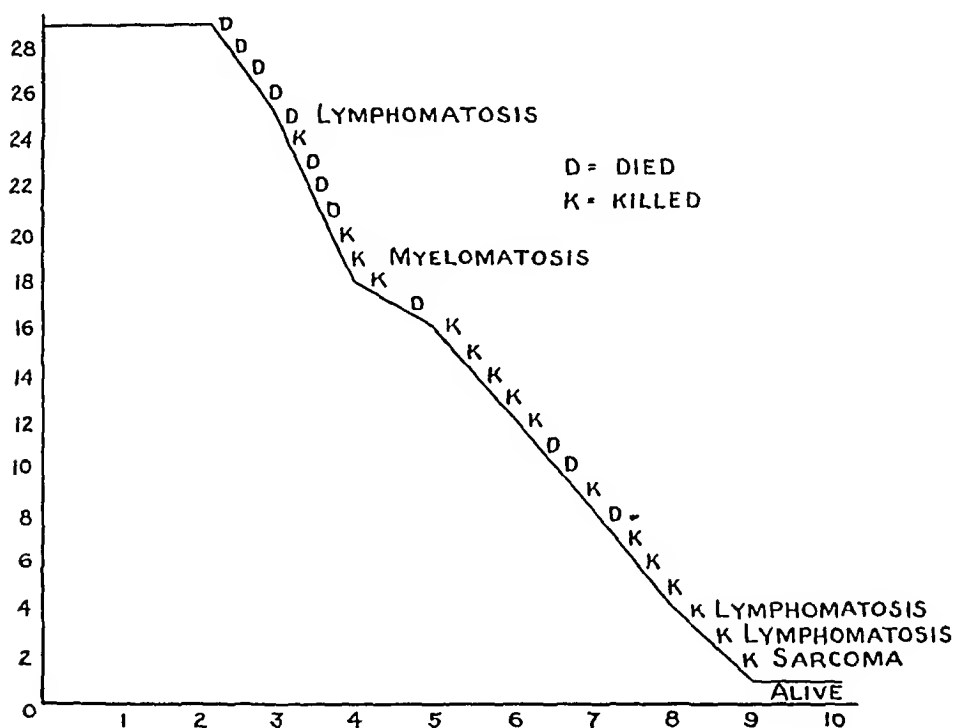


Fig 16—The number of the un inoculated control chickens in relation to the period of observation and to the spontaneous occurrence of lymphomatosis, myelomatosis and sarcoma. The vertical line of figures gives the numbers of chickens alive at the close of the periods of observation in months, shown in the horizontal line of figures. For example, after three months twenty-five of the twenty-nine chickens were alive and none of the four that had died (D) had leukosis or sarcoma.

nine control birds within this period of time. Experiments on neurolymphomatosis were terminated after approximately five months, and only one instance of lymphomatosis and one instance of myelocytomatosis were found among the un inoculated control chickens within this time. Many of the control chickens were killed after five months, but of the twelve chickens that were kept longer than six months two presented lymphomatosis and one sarcoma. We noted on previous

occasions the high incidence of similar neoplasms among chickens used in studies on active immunity to leukosis and kept in the laboratory during a period of from one to two years

INCIDENCE OF NEUROLYMPHOMATOSIS AMONG CHICKENS INOCULATED WITH TRANSMISSIBLE STRAINS OF LEUKOSIS AND SARCOMA

These birds were kept in the same animal room that housed the chickens inoculated with tissues of paralyzed birds, but usually in different cages From November 5 to April 27 a weekly record was kept

TABLE 14—A Periodical Record of the Incidence of Neurolymphomatosis Among the Chickens in Our Animal Rooms

Date	Neurolymphomatosis Strains 5, 6, 4, 14		Leukosis Strain 2		All Other Strains of Leukosis and Sarcoma		Total Number of Chickens in Animal Room
	Chickens in Experiment	Incidence of Neuro lymphoma tosis	Chickens in Experiment	Incidence of Neuro lymphoma tosis	Chickens in Experiment	Incidence of Neuro lymphoma tosis	
Nov 5-18	31	7	65	1	122	3*	218
Nov 19 Dec 9	42	2	62	2	133	1*	237
Dec 17 30	48	3	62	0	112	1†	222
Dec 31 Jan 13	44	5	52	0	99	0	195
Jan 14 27	67	4	72	0	112	1*	251
Jan 28 Feb 10	66	4	67	2	118	0	251
Feb 11 24	71	3	63	0	103	0	237
Feb 25 March 10	106	5	62	1	104	1‡	272
March 11 24	101	7	59	0	111	0	271
March 25 April 7	87	8	60	1	107	1†	279
April 7 21	72	11	53	0	99	0	224
Aver no chickens in animal room	67	5.1 (7.6%)	62	0.6 (1.0%)	111	0.7 (0.6%)	240

* In chicken inoculated with strain 1
† In chicken inoculated with strain 12
‡ In chicken inoculated with strain 13

of the number of animals in the laboratory and of the number of chickens with either clinical paralysis or thickening of nerves due to lymphomatous infiltrations This record, computed for approximately biweekly intervals (table 14), gives a fair estimate of the incidence of paralysis in our laboratories

Table 14 demonstrates that neurolymphomatosis is transmissible by inoculations with tissues from paralyzed chickens, but it also shows that paralysis occurs among chickens inoculated with tissues that were derived from seemingly unparalyzed birds

The chickens in the first group in table 14 were inoculated with material derived from paralyzed chickens Neurolymphomatosis strains 5 and 6 have been described Strain 4 was a transmissible strain of lymphomatosis that originated in a chicken unsuccessfully inoculated with tissues of Hodgkin's disease of man After four

successive subpassages this strain was dropped. Lymphomatosis of this strain was in most instances associated with extensive infiltration of peripheral nerves and occasionally with leukemic blood pictures. It produced infiltration in the inoculated breast muscle. Strain 14 originated in a chicken that was inoculated with sarcoma tissue occurring in an uninoculated control chicken. This strain, too, was discarded after three successive passages, mainly because the disease that developed among its passages was similar to that produced by strains 5 and 6.

The chickens in the second group in table 14 were inoculated with strain 2, known to produce lymphomatosis with microscopic infiltration in the nerves.

The third group comprises all other chickens kept in the animal room including those inoculated with strains of leukosis and sarcoma that do not produce paralysis and a few uninoculated chickens. Five of the eight instances of paralysis recorded in this group occurred in chickens inoculated with strain 1. Last fall a small "epidemic" of neurolymphomatosis occurred among the chickens inoculated with this strain for reasons that are unknown. Most of the cases occurred in a group of eight chickens that received fractions of plasma obtained by ammonium sulphate precipitation at various hydrogen ion concentrations. It is uncertain whether the four instances of lymphomatosis were spontaneous, or whether the plasma of the donor carried the agent of neurolymphomatosis. Since January this year no instance of paralysis has been seen in chickens inoculated with strain 1, and the severe test of intraneural inoculation, the results of which have already been described, indicates that this strain does not produce neurolymphomatosis.

COMMENT

Two varieties of transmissible lymphomatosis are described in this report. One (strain 2) is characterized by large lymphocytes, produces lymphomatous tumors after intramuscular transmission, infiltrates the blood-forming organs, and is almost invariably associated with lymphatic leukemia. It almost invariably produces severe anemia, often infiltrates nerves, but seldom produces clinical paralysis. It is very rare as a spontaneous disease and is readily transmissible by cell-free material. The second type of transmissible lymphomatosis (strains 5 and 6, neurolymphomatosis) produces mainly lymphomatosis of the nerves, often associated with lymphomatosis of the viscera, seldom with lymphatic leukemia and never with anemia. It is characterized by a predominance of small lymphocytes and is frequent as a spontaneous disease, but its transmissibility by cell-free material is thus far unproved.

Strain 2 can be propagated readily by cell grafts, and only under exceptional conditions can the free virus stimulate normal cells to neoplastic growth. An ultramicroscopic cause of lymphomatosis of strains 5 and 6 is still unproved. Nevertheless, analogy with related diseases of fowls suggests that neurolymphomatosis is also a neoplasm produced by a filtrable virus. The disease has the histologic characteristics of a neoplasm. It occurs almost exclusively in young adult chickens. It is a very common disease, its incidence being, in our experience, greater than that of all other neoplasms together. The

transmitting agent circulates in the blood of paralyzed chickens in high concentration throughout the entire course of illness even though the disease is not associated with leukemia. Ease of transmission by material containing live cells and difficulty of transmission in the absence of these cells are characteristic of many chicken sarcomas as well as of chicken leukosis.

Recent suggestions²¹ that all types of lymphoid leukosis or all types of leukosis including lymphomas are caused by a single virus are contradicted by our experience. Strains 1, 2 and 5 have been propagated side by side during the past year and introduced by similar routes of inoculation into experimental birds of the same age, breed and source, yet each strain retained its distinguishing features as shown in table 15.

TABLE 15—*Comparison of Observations on Strains 1, 2, 5 and 6*

Points of Comparison	Strain 1	Strain 2	Strains 5 and 6
Paralysis	None	None or slight	Conspicuous
Infiltration of nerves	None	Slight	Extensive
Blood changes			
(a) Red cell system	Erythroleukosis	Anemia	None
(b) White cell system	None or myeloblastic leukemia	Increase of large lymphocytes and occasionally of myelocytes	None or increase of small lymphocytes
Bone marrow	Hyperplastic	Hyperplastic	Usually normal
Site of intramuscular inoculation	No alteration detected	Infiltrated by large lymphocytes	No alteration detected
Inoculated nerve	Normal	Thickened	Thickened

The suggestion that all agents of leukosis may produce sarcoma is likewise erroneous. Among approximately three hundred chickens used in the study of neurolymphomatosis, only one instance of sarcoma of the type described by Rous was observed. This sarcoma proved to be readily transmissible (strain 15), but it did not produce neurolymphomatosis.

It is difficult to preserve strains of neoplasms by intravenous passages in animals that are likely to develop similar diseases spontaneously.

Subpassages of neurolymphomatosis are best made by intraneural inoculation. The early recognition of the disease by means of the characteristic local infiltration facilitates the maintenance of the strain. Strain 2 is best carried on by intramuscular inoculations. Diffuse infiltration or tumors formed by large lymphocytes in the inoculated muscle and the subsequent leukemia aid in the diagnosis of this strain. Strain 1 is best maintained by intravenous injection of blood into young chickens, thus far it is the only strain of leukosis the agent of which has been readily preserved in the dry state.

Many variations of transmissible lymphomatosis are known in mice, including one that often causes paralysis,^{30a} but lymphomatosis of numerous nerves and ganglions with slight or with no alteration in the blood-forming tissues is a unique phenomenon observed only in chickens. Neurolymphomatosis is very common among chickens throughout the world, but there is utter ignorance of the circumstances under which the spontaneous disease originates. The causation and pathogenesis of the disease are still obscure. If it may be assumed that the disease is a neoplasm produced by a filtrable agent, this agent may be either neurotropic or lymphocytotropic or both. If the malignant lymphocytes that infiltrate the nerves arise in the lymphoid tissues, why do they not form lymphomatous infiltrations in these tissues? If the assumed virus becomes attached to the nerve and attracts lymphocytes, why did we fail to produce lymphoid infiltration in the nerves inoculated with cell-free virus? If the cells are malignant lymphocytes that contain no virus, what are the natural means that bring about the malignant transformation of lymphocytes, endowing them with special affinity for nerve tissues?

V SUMMARY AND CONCLUSIONS

The virus of leukosis strain 2 brings about neoplastic growth of lymphocytes with characteristic morphologic and biologic properties. Injected intramuscularly it produces no tumors at the site of injection. The neoplastic cells produced by it in the blood-forming organs behave like the leukemic lymphocytes of mammals and can be grafted readily in muscle tissue, where they undergo autonomous multiplication and produce tumors.

Two transmissible strains of neurolymphomatosis (strains 5 and 6) that can be readily passed from diseased to healthy chickens by an inoculum containing viable lymphocytes are described. The transmitting agent became inactivated by freezing at -30°C for thirty minutes or by drying from the frozen state.

The blood of paralyzed chickens contains the transmitting agent in high concentration during the entire course of illness, and when introduced by the intravenous route it produces neurolymphomatosis indistinguishable from the spontaneous disease. Blood cells readily transmit the disease, but plasma does not. The transmissibility of the disease by a virus, though probable, is not proved.

After intraneural injection of tissues from paralyzed birds the inoculated nerve becomes greatly thickened, and general neurolymphomatosis follows. The disease spreads along the course of the nerve fibers, but the cord is seldom infiltrated, and distant lesions are probably the result of hematogenous spread.

Lymphomatous infiltration and tumors of the viscera may be caused by the same virus that produces neurolymphomatosis

Neurolymphomatosis is usually unassociated with morphologic blood changes, but in rare instances it is associated with lymphoid leukemia

Neurolymphomatosis is a neoplastic disease allied to leukosis and sarcoma, but it is not produced by the agent that causes erythroleukosis and myeloid leukosis, neither does the agent of neurolymphomatosis produce erythroleukosis and myeloid leukosis

Case Reports

GUMMA OF THE HEART

REPORT OF TWO CASES

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NEW YORK

Gumma of the heart undoubtedly existed unrecognized for centuries. Because of the disinclination of early nineteenth century physicians to perform necropsies in cases of inveterate syphilis,¹ it was not until 1845 that the first positive report of cardiac gumma was published, that by Ricord,² a syphilodermatologist.

Soon after this, Virchow³ divided tertiary syphilis of the heart muscle into fibrous and gummatous types. The former is generally held to be indistinguishable from diffuse myofibrosis found with coronary sclerosis, although Takata⁴ stated his belief that the syphilitic type can be recognized histologically. I am concerned here neither with the fibrous type of myocardial syphilis nor with the unconfirmed lesions described by Warthin⁵ but with the gummatous type, which constitutes a distinct anatomic entity.

Gummatous myocarditis may be diffuse or localized and occurs in congenital as well as in acquired syphilis.

DIFFUSE GUMMATOUS MYOCARDITIS

Diffuse gummatous myocarditis unassociated with grossly recognizable gummas is extremely rare, only seven cases could be found recorded in the literature.⁶ It is more often found in combination with

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1 Wilks, Samuel. Biographical Reminiscences, London, Adlard & Son, 1911 p. 17.

2 Ricord. *Gaz. d. hôp.*, Aug. 30, 1845, no. 101, p. 402.

3 Virchow, R. *Virchows Arch. f. path. Anat.* **15**, 217, 1858.

4 Takata, F. *Virchows Arch. f. path. Anat.* **228**, 426, 1920.

5 Warthin, A. S. *Am. J. Syph.* **2**, 425, 1918.

6 (a) Adler, I. *New York M. J.* **68**, 577, 1898 (cases 5 and 6). (b) Thorel, C. *Virchows Arch. f. path. Anat.* **158**, 271, 1899. (c) Busse, O., and Hochheim, W. *Arch. f. Ophth.* **55**, 222, 1903. (d) van Huellen, A. *Ztschr. f. Heilk.* **6**, 227, 1905. (e) Berblinger, W. *Zentralbl. f. allg. Path. u. path. Anat.* **21**, 1045, 1910. (f) Salles, P. H. E. *Contribution à l'étude anatomo-pathologique de la pancardite syphilitique*, Thèse de Paris, Paris, Ollier-Henry, 1918 (case 3).

the localized type (gumma) It is characterized by the presence of extensive interstitial and perivascular granulation tissue infiltrated by lymphocytes and plasma cells Occasionally giant cells are present There are also obliterative endarterial lesions and frequently milium areas of coagulation necrosis These necrotic foci are sometimes of submiliary size, but when larger constitute a stage of transition to the well localized gumma The myocardial fibers may show atrophy and fatty change There may be no macroscopic lesions or, at most, innumerable whitish flecks

The diagnosis can be made only by microscopic examination Because of the inconstant appearance of necrosis and giant cells and the frequent difficulty in differentiating less characteristic lesions from those of tuberculous etiology, Saltykow ⁷ and Baumgartner ⁸ placed the lesions of diffuse gummatous and tuberculous myocarditis in the same group and designated them as "specific productive myocarditis" Spirochetal stains are generally negative in tertiary syphilis and, therefore, often of no practical aid in diagnosis

LOCALIZED GUMMATOUS MYOCARDITIS (CARDIAC GUMMA)

The localized type of gummatous myocarditis known as the gumma is decidedly more common and characteristic, possessing the same appearance and structure as gummas elsewhere in the body A total of ninety-seven authentic cases of cardiac gumma have been reported ⁹

7 Saltykow, S. *Verhandl d deutsch path Gesellsch* **17** 321, 1914

8 Baumgartner, H. *Frankfurt Ztschr f Path* **18.91**, 1916

9 (a) Dandridge, N. P. *M & S Reporter* **28** 352, 1873 (b) Adler ^{6a} (case 7) (c) Luce, H. *Deutsches Arch f klin Med* **74** 370, 1902 Fahr, T. *Virchows Arch f path Anat* **188** 562, 1907 (d) Stockmann, W. *Ueber Gummiknoten im Herzfleische bei Erwachsenen*, Wiesbaden, J. F. Bergmann, 1904 (collected fifty-six cases, including four of his own) (e) Handford, H. *Brit M J* **2** 1745, 1904 (f) Keith, A., and Miller, C. *Lancet* **2** 1429, 1906 (g) Ashton, T. G., Norris, G. W., and Lavenson, R. S. *Am J M Sc* **133** 28, 1907 (h) Huchard, H., and Fiessinger, N. *Rev de med, Paris* **27** 948, 1907 (i) Robinson, G. C. *Bull Ayer Clin Lab, Pennsylvania Hosp* **4** 1, 1907 (j) Vaquez and Esmein. *Presse med* **15** 57, 1907 (k) Heineke, A., Muller, A., and von Hosslin, H. *Deutsches Arch f klin Med* **93** 459, 1908 (l) Handwerch, C. *Munchen med Wchnschr* **56** 916, 1909 (m) Klages. *ibid* **59** 1323, 1912 (n) Letulle, M. *Bull Soc anat de Paris* **87** 31, 1912, *ibid* **89** 402, 1914-1919 (o) McWeeney, E. J. *Tr Roy Acad Med, Ireland* **31** 413, 1913 (p) Lombardo, G. *Pathologica* **6** 83, 1914 (q) Soprana, F., and Piazza, C. *Riforma med* **31** 537, 567 and 590, 1915 (two cases) (r) Holterdorf, A. *Munchen med Wchnschr* **63** 1651, 1916 (s) Reinhardt. *ibid* **64** 1467, 1917 (t) Husche, K. *Ein Fall von Gummosis des Herzens*, Inaug. Dissert., Berlin, 1918 (u) Salles ^{6f} (case 1) (v) Bridgman, E. W., and Schmeisser, H. C. *Johns Hopkins Hosp Rep* **18** 90, 1919 (w) Takata ⁴ (four cases) (x) Girdwood, R. L. *M J South Africa* **16** 183, 1920-1921 (y) Spalding, E. D., and Von Glahn, W. C.

The gumma may be solitary, but usually it is multiple and nodular, although serrated and jagged surfaces may be seen. It varies from pin-head size to that of a fowl's egg and is usually dull yellowish or grayish white, often with a bacon-like appearance. Palpation gives a firm, elastic, rubber-like sensation, from which the gumma derives its name (*Gummgeschwulst*). The older, more chronic lesion is generally surrounded by an irregular dense fibrous capsule which contributes to the firmness of the entire nodule.

The most frequent site of the cardiac gumma is the left ventricular myocardium, particularly the basal portion of the interventricular septum. Gummas in this location often give rise to heart block. Interference with cardiac function also arises when gummatous lesions compromise or invade the valves (table). A number of cases of involvement of the papillary muscles have been reported,¹⁰ in one instance with

Bull Johns Hopkins Hosp **32** 30, 1921 (*z*) Major, R H Arch Int Med **31** 857, 1923 (*aa*) Friedman, W Proc New York Path Soc **24** 24, 1924 (*bb*) Young, W A Tr Roy Soc Trop Med & Hyg **19** 87, 1925-1926 (*cc*) Cabot, R C Facts on the Heart, Philadelphia, W B Saunders Company, 1926, p 375 (*dd*) de Marval, L, and Vivoli, D Rev Soc argent de biol **2** 425, 1926, Rev Soc de med int y soc de fisiol **2** 397, 1926 (*ee*) Cleland, J B M J Australia **14** 540, 1927 (*ff*) Jansen, H Virchows Arch f path Anat **264** 730, 1927 (three cases) (*gg*) Cookson, H Brit M J **2** 94, 1929 (*hh*) Hajoshi, I Ztschr f Kreislaufforsch **21** 34, 1929 (*ii*) Staemmler Verhandl d deutsch path Gesellsch **25** 262, 1930 (*jj*) Kun, E Ztschr f Kreislaufforsch **24** 1, 1932

In addition, twenty cases of cardiac gumma have been found in which a description is absent or inadequate. Goodhart, cited by Hall, D G Edinburgh M J **14** 322, 1903 Renvers, R Therap d Gegenw **6** 433, 1904 (three cases) Schmorl Munchen med Wchnschr **54** 285, 1907 (two cases) Brooks, H Am J M Sc **146** 513, 1913 (five cases) Welch, cited by Cabot^{9cc} Clawson, B J, and Bell, E T Arch Path **4** 922, 1927 (three cases) Coombs, C F Lancet **2** 227, 281 and 333, 1930 Martland, H S Am Heart J **6** 1, 1930-1931 Warthin, A S ibid **6** 163, 1930-1931 (two cases) Army M Museum, Washington, D C, Accession no 26893, courtesy of Major V H Cornell, Medical Corps, U S A

There have also been reported a number of instances of cardiac gumma which lack sufficient criteria for inclusion among the authentic cases. Israel, O Berl klin Wchnschr **32** 792, 1895 von Genersich, A Pest med-chir Presse **33** 84, 108, 1897 Jagic, N Ztschr f klin Med **66** 183, 1908 Rosenfeld, F Deutsches med Wchnschr **40** 1044, 1914 Macfie, J W S, and Ingram, I Ann Trop Med **14** 147, 1920 Morin, H G S, and Fabre, H Marseille-med **60** 1430, 1923

¹⁰ Burney, Yeo, quoted by Phillips, S Lancet **1** 223, 1897 Cayley, W Tr Path Soc London **26** 32, 1875 Jurgens Berl klin Wchnschr **28** 1031, 1891 Rolleston, H D Tr Path Soc London **44** 37, 1893 Lorrain Bull Soc Anat de Paris **70** 693, 1895 Stockmann^{9d} (case 2) Ashton, Norris, and Lavenson^{9g} Spalding and Von Glahn^{9j}

rupture and sudden death^{9b} The endocardium or the epicardium overlying a subjacent gumma may show localized nonspecific sclerotic thickenings, infrequently, however, they may be involved by the specific gummatous process In rare instances partial obliteration of the pericardial cavity may be produced

The fate of a gumma in the heart is identical with that of gummas in other organs Because of a marked tendency to fibrosis it may heal entirely, occasionally with calcification More rarely it may undergo extensive softening with the formation of cavitations, in which event communication with the endocardial chambers is frequently established and cardiac aneurysm results¹¹ A reported instance of embolization from such a source could not be confirmed¹² Communication with the pericardial cavity is rarer and invariably associated with a complete rupture of the cardiac wall through the aneurysmal portion, this is manifested by sudden death¹³ Perforation of the interventricular septum by a gumma has been reported in congenital syphilis,¹⁴ but the pathologic diagnosis is not conclusive

The histologic diagnosis of cardiac gumma is based on the characteristic finding of a more or less round central necrotic area containing nuclear and cytoplasmic debris and occasionally remnants of myocardial fibers The last point has been stressed in the literature as being of diagnostic aid in differentiating it from the tubercle Surrounding this central area of coagulation necrosis there is a fairly well defined zone of granulation tissue, usually densely infiltrated by lymphocytes, plasma cells and fibroblasts Giant cells, occasionally of the Langhans type, epithelioid cells and frequently eosinophilic leukocytes may be seen Lesions of the vessels in this zone may be entirely absent More frequently endarterial or endophlebitic hyperplastic processes, often with perivascular accumulations of round cells, are evident In larger and more chronic lesions the entire inflammatory focus is usually, in turn, surrounded by a dense connective tissue capsule, from which fibrous strands radiate between the bundles of adjacent myocardial fibers The gumma as a whole has a pronounced fibrous aspect

11 (a) McNalty, G W M Times & Gaz 1 624, 1873 (b) Goodhart, J F, and Green, A W Tr Path Soc London 38 102, 1887 (c) Pitt, G N *ibid* 42 61, 1891 (d) Kockel, R Arb a d med Klin zu Leipzig 1 294, 1893 (e) Kronig Berl klin Wchnschr 32 969, 1895 (f) Stolper, P Bibliot med (pt C) 6 25, 1896 (g) Jodlbauer, A Ein Fall von Syphilis des Herzens, Inaug Dissert, Munich, 1897 (h) Duckworth, D Tr Clin Soc London 29 7, 1896 (i) Dandridge^{9a} (j) Luce^{9c} (k) Klages^{9m} (l) Young^{9bb} (m) Cookson^{9gg}

12 Oppolzer, quoted by Lang, T Die Syphilis des Herzens, Vienna, Wilhelm Braumuller, 1889

13 Goodhart and Green^{11b} Pitt^{11c} Kronig^{11e} Dandridge^{9a}

14 Hughes, W E Proc Path Soc, Philadelphia 3 17, 1899-1900

Cardiac gummas are rarely diagnosed clinically, although their presence can occasionally be suspected. At times, the lesion is an accidental postmortem finding which caused no symptoms during life. The following cases illustrate these points.

REPORT OF CASE 1

Gummas of the Left Ventricular Myocardium and Interventricular Septum, Necrosis, Cavitation and Communication with the Left Ventricular Cavity, Sacculi Protrusion into the Pulmonary Subvalvular Region Producing Pseudo-stenosis

A 53 year old Italian man was admitted to the service of Dr B. S. Oppenheimer on Dec 8, 1931, complaining of weakness, cough, anorexia, a pressing sensation in the left lower part of the chest radiating to the left upper part of the chest on walking, dyspnea on exertion and swelling of the ankles. The symptoms were of three months' duration.

There was slight cyanosis of the finger-tips and lips. The patient was mildly orthopneic. The temperature was 98.8 F, the pulse rate 116, and the respiratory rate 28 per minute. The pupils were regular and equal and reacted to light and in accommodation. There was dulness at the extreme base of the right lung with moist rales at the bases of both lungs. The heart was enlarged toward the axilla and extended about 5 cm. to the left of the sternum in the second intercostal space. The right border was percussed 4 cm. to the right of the sternum. At the base of the heart and just to the left of the sternum, there could be felt a strong systolic impulse fading in intensity toward the apex, where it was practically impalpable. A loud, sharp systolic first sound and a whistling systolic murmur were audible in the same area with the same distribution of intensity. At the apex there was a short systolic bruit transmitted into the upper part of the axilla and around into the left interscapular region. The second cardiac sound was barely audible. Occasional interpolated cardiac beats were present, the rhythm being otherwise regular. The pulses were equal, synchronous and of good quality. The liver was felt 4 fingerbreadths below the right costal margin, its edge was soft and round. Edema of the feet was present. Several serpiginous and round, paper-thin scars were observed over the right calf. The neurologic status was negative.

The Wassermann and Kahn reactions of the blood were both positive (4+). The systolic blood pressure was 120 mm. of mercury and the diastolic 65. The venous pressure was 9.5 cm. of blood (direct method). The hemoglobin was 100 per cent (17 Gm. per hundred cubic centimeters of blood). The leukocyte and differential counts were normal. The blood urea nitrogen was 42 mg. per hundred cubic centimeters. The urine contained a small amount of albumin.

A roentgenogram of the chest (fig 1A) showed a marked enlargement of the heart to the right and left. A prominence, roughly the size of a large plum, was evident in the region of the pulmonary artery.

An electrocardiogram revealed the rhythm to be very irregular owing to a combination of occasional sinus block with numerous nodal beats. The main deflection was very low, notched and widened (0.12 second) in all leads and partly inverted in lead III. The T-wave was low in all leads and inverted in leads I and II. The curve was thought to be suggestive of extensive myocardial damage with disturbance of the pacemaker. Three tracings showed essentially the same abnormalities.

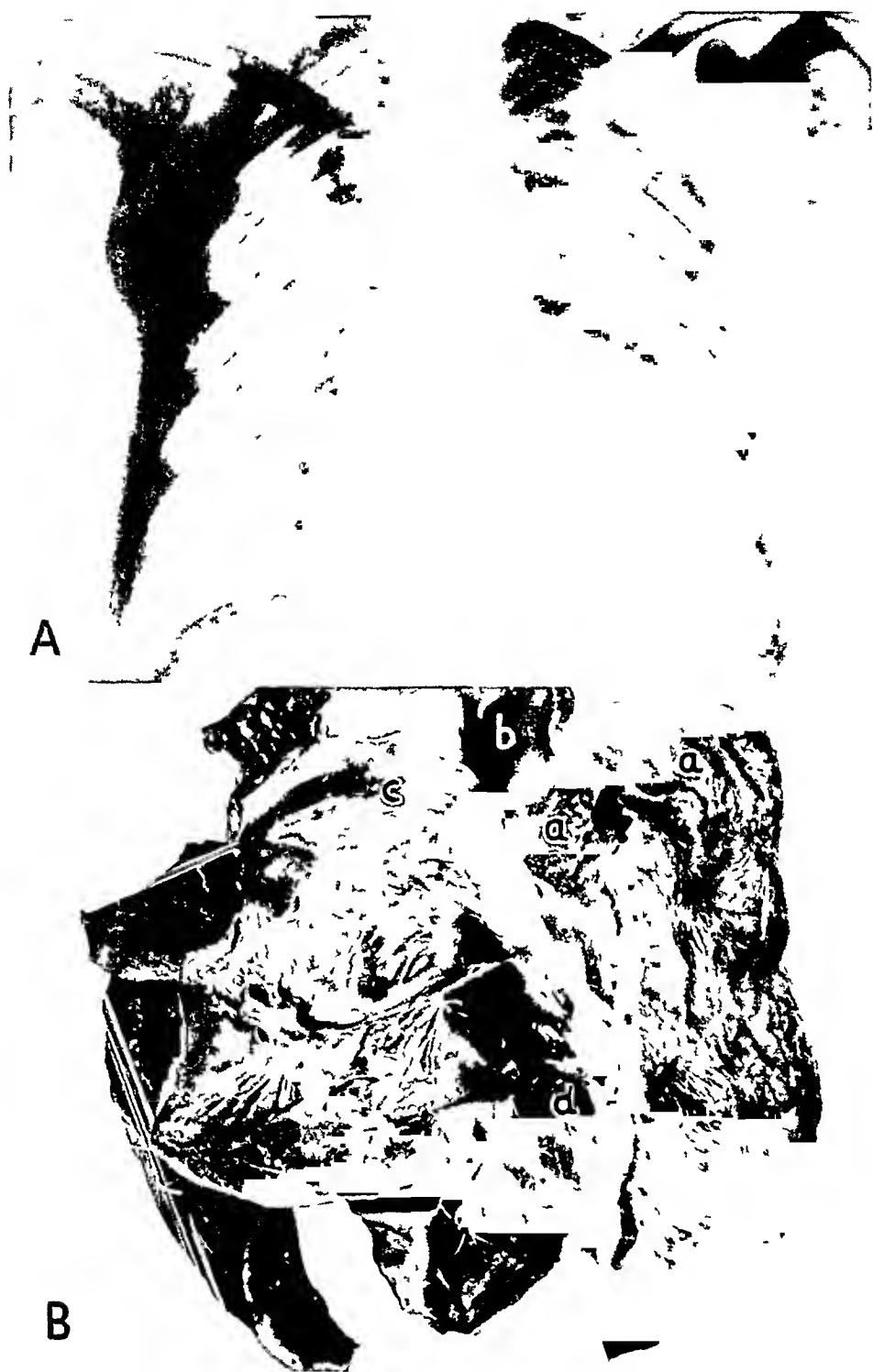


Fig 1 (case 1) —*A* roentgenogram of the chest, showing marked enlargement of the heart to the right and left. The large prominence in the region of the pulmonary conus was shown to be due to a gummatous aneurysm at the base of the left ventricle. *B*, anterior view of the heart. Note the large saccular eminence (*a*) subjacent to the pulmonary conus (*b*) and protruding well into the pulmonary subvalvular region (*c*), creating the effect of a pseudostenosis. Near the apex on the septal wall is a firm yellowish myocardial gumma (*d*).

Despite diuretic and bismuth therapy, the patient grew steadily worse and died suddenly on the nineteenth day after admission

The final clinical impressions included cardiac insufficiency, aortic stenosis, syphilis and a congenital cardiac lesion

Autopsy—The heart weighed 520 Gm. It was irregularly enlarged and somewhat distorted (figs 1 *B* and 2). The visceral pericardium, particularly over the left ventricle and to a lesser extent over the right ventricle, had undergone a yellowish, somewhat nodular transformation. The most conspicuous distortion in the shape of the heart was due to the appearance of an irregularly rounded protuberance which appeared to be a continuation of the upper part of the anterior surface of the left ventricle. This protuberance was somewhat pear-shaped, and its apex extended approximately 3 cm below the auriculoventricular sulcus anteri-

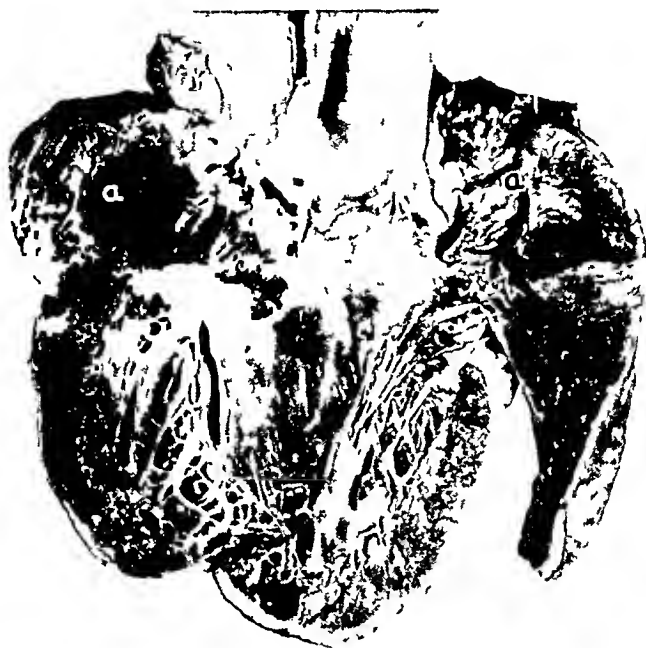


Fig 2 (case 1)—View of the left side of the heart showing the large trabeculated communicating aneurysmal cavities (*a*) at the base of the left ventricle and anterior portion of the interventricular septum. The aorta grossly shows no lesions suggestive of syphilis, although there is definite microscopic evidence of such involvement at the root

orly. Its upper portion reached approximately 4 cm above the sulcus, abutting against the left lateral surface of the pulmonary conus. The left border of the pear-shaped mass extended over the margo obtusus and abutted against the left auricular appendix, which was raised by it to an upright perpendicular position.

The right ventricle was dilated. The columnae carnae were atrophic and flattened. The endocardium was somewhat thickened and whitened. Near the apex (on the septal surface) of the right ventricle was a shiny, yellowish, firm mass about the size of a hazelnut, shining through the endocardium. The outflow tract of the right ventricle was enlarged and presented a saccular aneurysm on its left wall. This aneurysm was the size of a pigeon's egg. It abutted against the left pulmonary cusp, extending 0.5 cm above it. The aneurysmal mass was

covered by rough endocardium and formed a definite obstruction to the right outflow tract. The pulmonary cusps and artery were normal.

The left ventricle was atrophic, the outflow tract was enlarged and showed irregular whitened endocardial areas. One of these (on the septal surface) overlay a hyaline connective tissue mass which merged with the gumma described in the right ventricle. The papillary muscles were normal. Immediately anterior to the right aortic cusp and situated on the interventricular septum was a large foramen which opened into the irregular pear-shaped prominence seen on the outer surface. This prominence was seen to consist of a sacculated aneurysmal dilatation of the left ventricle, one of the sacculi forming the aneurysmal bulging which obstructed the right outflow tract. The sac was, on the whole, smooth and lined with endocardium. Portions of it, however, presented a thin, furrowed thrombotic covering. The right portion of the left aortic cusp was impinged on and involved by the aneurysmal bulging. The remainder of the cusp was normal. The right and posterior aortic cusps were normal.

The aorta showed mild atheromatous changes but no lesions characteristic of syphilis. The orifice of the left coronary artery was somewhat narrowed, that of the right coronary artery, markedly narrowed. The major coronary arteries, the right more than the left, presented slight atheromatous lesions without narrowing. Both auricles and auriculoventricular valves were normal.

Microscopic Examination—Aorta. The root of the aorta near the left-right commissure was the site of an advanced adventitial and periaortic inflammatory lesion. The walls of the vasa vasorum were extremely thickened, chiefly by intimal hyperplasia, which in many caused great narrowing of the lumen. Diffuse and perivascular infiltrations of small round cells were noteworthy. The annulus fibrosus and the tissue behind it were markedly fibrosed, thickened and similarly involved by the inflammatory process, having a position adjacent to the fibro-necrotic wall of the aneurysmal cavity at the base of the left ventricle. The periaortic subepicardial fat tissue in the angle between the root of the aorta and the aneurysmal wall was likewise implicated in the infiltrative process (see Epicardium). The media of the aortic root was relatively intact. There was slight fibro-elastic proliferation of the intima.

Left Aortic Cusp. This leaflet at its junction with the wall of the aneurysm was the site of an interstitial valvulitis consisting of diffuse capillarization and infiltration with lymphocytes, large mononuclear cells and fibroblasts, which extended uninterruptedly from the base through the entire length of the leaflet. The structure was tremendously thickened, chiefly by the addition of an inflamed broad fibro-elastic band of tissue lying adjacent to the hyperplastic ventricular layer of elastica. The latter, as well as the original fibrous and spongy layers, was likewise involved in the inflammatory process, which was obviously an extension from that of the contiguous structures at its root.

Wall of Aneurysm at Base of Left Ventricle. On the internal surface was an adherent thrombus undergoing organization at its base. In the center of the wall was a large, more or less circular, homogeneous area undergoing coagulation necrosis. Surrounding it was a narrow zone densely infiltrated with lymphocytes, plasma cells, large mononuclear cells and fibroblasts. External to this was a dense fibrous capsule which, in one area, was the site of marked capillarization and formation of granulation tissue (fig 3A). Giant cells were absent. The Levaditi stain was negative.

Another portion of the wall disclosed an older, more fibrotic lesion characterized by many small focal and confluent areas of coagulation necrosis situated in the interstices of an extensively sclerotic, infiltrated and capillarized band of tissue.

In the angle at the junction with the dome of the left ventricular myocardium, the fibrous aneurysmal wall diffused into the subepicardial fat tissue, giving the appearance of an extensive granulomatous lesion, replacing the fat cells and containing numerous thick-walled vessels with endarterial lesions, dense focal and diffuse accumulations of round cells and fibroblasts and excessive fibrous tissue proliferation. An occasional lymphatic channel was loaded with lymphocytes.

Epicardium The subepithelial connective tissue layer of the epicardium was markedly thickened and fibrotic. The superficial zone of the subepicardial fat tissue (fig 3 *B*) was massively infiltrated by lymphocytes, plasma cells, large mononuclear cells and fibroblasts, with an occasional giant cell. This layer was vascularized by excessive numbers of young and old, thick-walled capillaries, with

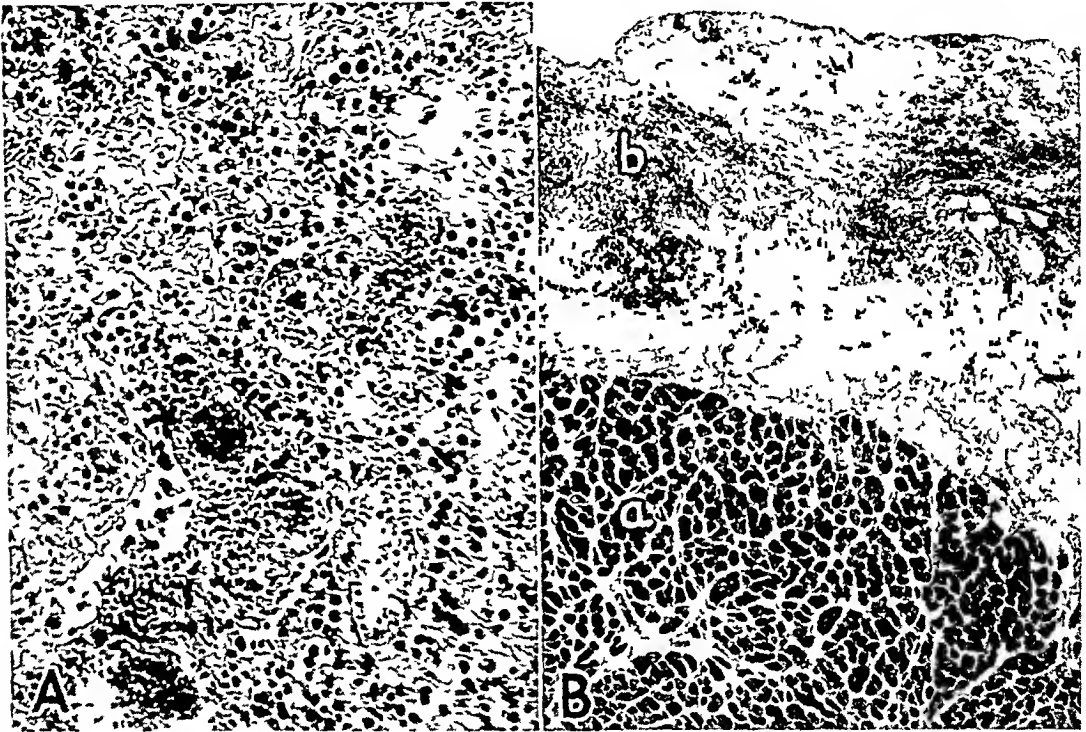


Fig 3 (case 1)—*A*, granulation tissue surrounding a gumma in the wall of the aneurysm, dense infiltration by lymphocytes, plasma cells, wandering cells and fibroblasts with numerous capillaries. Hematoxylin and eosin, obj 16 mm, $\times 200$. *B*, right ventricle (*a*) with overlying epicarditis (*b*). The granulomatous process consisting of dense round cell infiltration, fibroblasts, numerous capillaries and proliferated connective tissue is seen to occupy chiefly the superficial portion of the epicardium. The underlying myocardium in this section is relatively normal. Hematoxylin and eosin, obj 25 mm, $\times 50$.

marked proliferation of the lining endothelial cells. The lymphatic channels were frequently packed with lymphocytes.

In general, the entire granulomatous lesion was situated at an appreciable distance from the underlying myocardium, although occasionally it dipped down to involve its surface. The myocardium of the right ventricle was extensively scarred. These large areas of fibrosis contained isolated muscle fibers, thick-walled vessels with intimal hyperplasia and narrow lumens and a scattering of lymphocytes.

Such a superficial scarred area was seen to send narrow fibrous strands into the epicardial lesion where they were in proximity.

Right Ventricle A section through the firm yellowish lesion at the base of the trabeculum septomarginalis disclosed an area of coagulation necrosis identical with that noted in the wall of the aneurysm. The appearance was typical of gumma.

Other Organs Significant pathologic observations in the other organs included chronic passive congestion of the liver, spleen and kidneys, a pulmonary infarct and fibrosis of the pancreas. Anatomic evidence of syphilis could not be demonstrated in any of the organs other than the heart.¹⁵

COMMENT ON CASE 1

The discovery of gummatous cardiac aneurysms in this patient was a complete surprise but explained the phenomena which were so puzzling during life, i e., the signs of valvular stenosis, a prominence in the region of the pulmonary artery (by roentgenogram) and serologic findings indicative of syphilis.

The necropsy disclosed a rare consequence of cardiac gumma, i e., caseation, cavitation and communication with the ventricular cavity. Thirteen instances of gummatous cardiac aneurysm have been observed.¹¹ (A number of so-called syphilitic cardiac aneurysms due to "syphilitic coronary endarteritis" have been excluded.) Lack of histologic detail and absence of investigation of the coronary system detract from the value of some of the earlier reports. Gummatous aneurysm, like the gumma, may be situated in any portion of the heart but almost always occurs in the left ventricle. In contrast to the usual apical position of cardiac aneurysm in consequence of coronary occlusion, the gummatous aneurysm, in the majority of cases, is found at the base of the ventricle behind either flap of the mitral valve or in the upper portion of the interventricular septum.

In this case the large saccular protuberance at the base of the left ventricle in the region of the margo obtusus (fig 1 *B*) accounts for the unusual roentgen shadow in that situation (fig 1 *A*). This contributed to the difficulty in arriving at a clinical diagnosis. The unusual cardiac physical signs together with the roentgen shadow presented a complex, rendered more enigmatic by the fact that the patient was syphilitic. It was difficult to correlate the latter finding with the observation of the maximum physical signs (strong systolic impulse, sharp first cardiac sound, and whistling systolic murmur) at the pulmonic area. A suspicion of a congenital cardiac lesion, such as pulmonic stenosis, was further strengthened by the absence of the second pulmonic sound, however, this was not compatible with the roentgen shadow in the region of the pulmonary artery. Nevertheless it was felt that the auscultatory cardiac signs were an expression of orificial stenosis, the exact situation of which was uncertain.

¹⁵ The brain was not examined.

Examination of the heart post mortem revealed this impression to be correct, although the mechanism was entirely unsuspected. Figure 1 *B* shows to what extent the gummatous aneurysm in the upper portion of the interventricular septum protruded into the region of the pulmonary valve to cause a pseudostenosis with consequent dilatation of the right side of the heart.

A number of cases have been recorded in the literature (table) in which obstruction in the outflow tract of the right ventricle was caused by a gumma protruding from the interventricular septum, but no other case could be found in which the syndrome of Beinhelm was simulated by a gummatous aneurysm. Other instances of valvular or subvalvular stenosis or insufficiency have been reported in which the valve or juxta-valvular region was directly invaded or compromised by a gummatous process in the contiguous myocardium or great vessels (table). Gummas in the upper portion of the interventricular septum protruding into each ventricular chamber and causing both aortic and pulmonic stenosis were found in a case reported by Major¹⁴. Similar biostial involvement was present in the cases of Luce¹⁵ (later reported by Fah¹) and Robinson¹¹. Because of the relative frequency of gummatous changes in the upper portion of the interventricular septum and root of the aorta, it is not surprising that interference with valvular function occurs most frequently at the aortic and pulmonic valve regions. From the tabulation it may be seen that the order of descending frequency of such interference is pulmonary, aortic, tricuspid and mitral. Acquired syphilis has not yet been proved to originate in a valve. Staemmler¹¹ pointed out that the gummatous process in the mitral valve in his case originated from the root of the aorta.

Of further pathologic interest in this case was the marked chronic epicarditis (fig 3 *B*), which was most evident at the base of the ventricles. This inflammatory granulomatous lesion was undoubtedly syphilitic and was seen to originate by extension from the gummatous wall of the aneurysms. A similar lesion, but lacking vascular changes, was noted by Thorel¹² in a case of diffuse gummatous myocarditis and was apparently the first of the type to be reported. Salles¹³ published excellent descriptions and photomicrographs of the characteristic lesion, which in his case arose from sclerogummatous nodules in the inter-aortopulmonary subepicardial region. "Syphilitic epicarditis" with miliary gummas was likewise described by Letulle¹⁰. Identical microscopic alterations of the epicardium were found in a case of congenital cardiac gumma by Oberhammer¹⁶.

16 Oberhammer, K. *Ztschr f Kreislaufforsch* 19 9, 1927

Macroscopically, the epicardium may be granular as well as thickened¹⁷ Girdwood¹⁸ observed a diffuse thickening of the epicardium extending upward into the adventitial tissue around the intrapericardial portion of the aorta and pulmonary artery There were no gross lesions of syphilitic aortitis in his case, although they were present in the cases of Salles and Letulle Lesions histologically similar to those observed in this case are seen not infrequently in the epicardium and adventitia at the root of the aorta in cases of syphilitic aortitis These lesions, too, undoubtedly represent extension from the inflamed aorta and occasionally are seen to extend a short distance down over the contiguous ventricular myocardium

It is apparent that in cases of cardiac gumma, chronic granulomatous epicarditis is of syphilitic origin even though foci of coagulation necrosis and giant cells are absent The visceral layer of the pericardium is involved by extension from contiguous myocardial (or aortic) gummas

REPORT OF CASE 2

Gummatous Aortitis with Commissural Involvement, Gummas of the Myocardium, Rheumatic Mitral and Aortic Stenosis and Insufficiency

The records in this case have been made available through the kindness of Drs Antopol and Weiss of the Bayonne Hospital and Dispensary, Bayonne, N J A Negro, aged 40, entered the hospital moribund on Sept 1, 1932, complaining of dyspnea, orthopnea, palpitations and swelling of the feet and abdomen for five months He had had polyarthritis fifteen years before admission Three years later he contracted a purulent urethral discharge and a sore on the penis Two years prior to admission he was given a series of intragluteal injections, presumably for syphilis

On examination he was dyspneic and orthopneic Râles were heard throughout both lungs A diffuse pulsation was present over the left lower part of the chest anteriorly The heart was enlarged to the left, its apex was in the sixth left intercostal space at the anterior axillary line, the right border of the heart was percussed 1 fingerbreadth to the right of the sternum, there were systolic and diastolic murmurs over the entire precordium The cardiac rhythm was completely irregular The liver was enlarged, and there were signs of free fluid in the abdomen Marked albuminuria was found The patient died two days after admission

Autopsy—Only the heart and aorta are described in detail The heart weighed 600 Gm and was enormously enlarged The right auricle was dilated The tricuspid valve showed fusion of the septal-anterior commissure The chordae tendineae to the anterior cusp were slightly thickened The right ventricle showed marked dilatation of the inflow and outflow tracts The pulmonary cusps were normal The pulmonary artery at the base of the right-left commissure was slightly thickened The pulmonary artery was somewhat dilated and delicately puckered and showed several small yellowish plaques

The left auricle was markedly dilated The endocardium was thickened The mitral valve showed marked universal thickening with stenosis of the orifice and fusion and thickening of the chordae tendineae When the mitral ring was pal-

¹⁷ Wagner, K E, and Qwiatkowski, G I Virchows Arch f path Anat
¹⁸ 171 369, 1903 de Marval and Vivoli 1904

pated, the posterior commissure presented a rubbery resistance to pressure. Sections through this region showed extensive scarring. In its center was a glistening, pale yellow, firm mass measuring 2 mm in diameter and reaching almost to the endocardial surface under the mitral pocket. The entire posterior mitral pocket was covered with a thrombus. Another typical gumma was disclosed 1 cm to the left of the one just described. The left ventricle was dilated and hypertrophied. The aortic cusps were thickened. The right-posterior and left-right commissures showed typical rheumatic fusion, but the left-posterior showed a typical syphilitic separation.

The aorta, starting with the insertion of the annulus and continuing up through the entire arch, showed porcelain-blue longitudinal wrinkling and puckering of the intima. The orifices of the coronary arteries were narrowed by the inflammatory process, the right more than the left. The coronary arteries were otherwise normal.

Microscopic Examination—Aorta The architecture was markedly distorted by an infiltrative and sclerotic process. The adventitia was tremendously thickened and fibrosed. There were marked obliterative endarterial proliferation of the vasa vasorum and dense diffuse and focal perivascular and perineural aggregations of lymphocytes and plasma cells. Large, irregular, dense fibrous scars, usually perivascular, were present in the media, with thickening, rupture and distortion of the elastic fibers. Innumerable capillaries with perivascular lymphocytic and plasma cell infiltrations were likewise found diffusely in the media. A few focal areas of necrosis with nuclear debris were situated in the outer third of the media. There was a noninflammatory dense fibro-elastic proliferation of the intima, which was most marked over the more advanced medial lesions.

Commissure Between Left and Posterior Aortic Cusps The aortic lesion just described continued unchanged into the region of the attachment of the two aortic cusps, which grossly showed separation. In addition in the annulus fibrosus, there were giant cells in moderate number, occasionally resembling the Langhans type.

Left Aortic Cusp The attached segment of aorta showed a lesion the same as that just described, descending into the wall of the sinus of Valsalva, but ending abruptly at the upper portion of the aortic annulus. The "ring spongiosa"^{17a} of the aortic cusp contained several smooth muscle bundles and many capillaries and arteries. The vessels ascended in liberal numbers throughout the length of the valve cusp in the broad fibro-elastic reduplication situated on the ventricular side of the hyperplastic elastica ventricularis. Toward the closure line a moderate infiltration with lymphocytes was present in the reduplicated layer.

Posterior Leaflet of the Mitral Valve The ring of the valve was fibrotic, vascularized and infiltrated with plasma cells and lymphocytes. The elastica auricularis at this point became remarkably hyperplastic, widened and deviated toward the ventricular side of the leaflet by broad hyperplastic fibro-elastic reduplications, themselves the site of marked vascularization and lymphocytic infiltration continuous with that at the ring.

Mitral Valve at Posterior Commissure A section through the yellow nodule in the valve pocket showed a typical blood platelet thrombus in the pocket undergoing basal organization. It was thickest where it overlay the superficially situated necrotic nodule. The latter had a fairly homogeneous appearance in the center, where it was comprised of cytoplasmic and nuclear debris. This mass of necrotic tissue was surrounded by a more or less irregular zone of granulation tissue con-

^{17a} Gross, L., and Kugel, M. A. *Am J Path* 7:445, 1931

taining many capillaries and dense infiltrations of lymphocytes, plasma cells, fibroblasts and histiocytes. Delicate fibrous strands emerged from the inner aspect of this zone to invade the central necrotic area.

External to the active granulating zone was a dense, jagged fibrous capsule with prolongations along the adjacent subendocardium and between the myocardial fibers. These strands were likewise infiltrated with lymphocytes and, to a lesser extent, with plasma cells. They contained vessels in abundance, which in the region of the auriculoventricular junction showed extreme intimal hyperplasia, often with complete obliteration of the lumens. There were no giant cells.

Left Ventricle There was a definite excess of perivascular fibrous tissue, as well as a slight, more diffuse interstitial fibrosis. No definite Aschoff bodies were found. There was moderate hyperplasia of the intima of the smaller and larger arteries, often localized in form. The epicardium was not thickened, but contained numerous foci of plasma cells and lymphocytes.

Pulmonary Artery and Valve The pulmonary artery was normal. The ring spongiosa contained a few blood vessels and no cells. There were no inflammatory changes in the valve.

Commissure Between Right and Left Pulmonary Valve The wedge of media at the root of the pulmonary artery which descends into the commissure was invaded by numerous blood vessels, which were surrounded in the outer zone by plasma cells. The adventitia and media were scarred and showed rupture and proliferation of the elastica. The valvular endocardium and intima in the commissural region were thickened by dense, vascularized scar tissue.

COMMENT ON CASE 2

This case represents the type in which cardiac gumma is an incidental finding at necropsy and has been unproductive of clinical manifestations. The site of the gumma was unusually firm to the touch. It was situated at the posterior commissure of the mitral valve, which was the site of rheumatic, sclerotic disease. On section, the firmness was found to be due largely to the dense, thick fibrous capsule which enveloped the gumma.

The history of this patient included both polyarthritis and a penile lesion. Either rheumatic or syphilitic aortic insufficiency could have accounted for the clinical picture of cardiac decompensation. The necropsy disclosed the fact that both these lesions were present.¹⁸

RECAPITULATION

Acquired tertiary syphilitic disease of the heart (exclusive of syphilitic aortitis with commissural involvement) is infrequent. If one omits from consideration the controversial diffuse fibrous type, the disease may be said to assume the form of diffuse interstitial gummatous myocarditis or that of localized gummatous myocarditis. The latter type (cardiac gumma) is by far the more common lesion. Direct exten-

¹⁸ The association of rheumatic valvulitis with (gummatous) commissural syphilis in the same valve is a very rare postmortem observation (Sager, R. V., and Sohval, A. R. *Arch. Path.* **17** 729, 1934).

*Cases in Which Cardiac Valves Were Compromised Either by Direct Gummatus
(or Granulomatous) Invasion or by Juxtaposed Gumma*

Author	Valve Invaded	Origin of Syphilitic Process	Valve Compromised by Juxtaposed Gumma	Origin of Gumma		
Wagner Arch d Heilk 7 518, 1866			Pulmonary	Interventricular septum		
Schwalbe Virchows Arch f path Anat 119 271, 1890	Pulmonary	Pulmonary artery	Pulmonary	Interventricular septum		
Volmer Ueber Gummata des Herzens, Inaug Dissert , Kiel, 1893 (case 2)			Tricuspid	Right ventricle		
de Massary Bull Soc anat de Paris 9 594, 1895			Aortic	Left ventricle		
Luce (Fahr) ^{9c}			Pulmonary and aortic	Interventricular septum		
Stockmann ^{9d} Case I A	Pulmonary	Interventricu- lar septum	Aortic	Interventricular septum		
Case III			Pulmonary	Interventricular septum		
Robinson ^{9l}	Aortic and tricuspid	Interventricu- lar septum	Aortic	Interventricular septum		
Klages ^{9m}	Aortic	Aneurysm of root of aorta, interventric- ular septum				
Holterdorf ^{9r}	Pulmonary	Pulmonary artery				
Bridgman and Schmeisser ^{9v}	Tricuspid	Interventricu- lar septum				
Girdwood ^{9x}			Pulmonary and tricuspid	Interventricular septum, right auriculovent- ricular septum		
Spalding and Von Glahn ^{9y}	Aortic (loss of substance)	Aorta				
Major ^{9z}	Aortic and pulmonary	Interventricu- lar septum	Pulmonary	Interventricular septum		
Friedman ^{9aa} Cleland ^{9ee}	Mitral	Aorta (?)	Pulmonary	Interventricular septum		
Gallavardin and Josselyn Lyon méd 139 135, 1927	Aortic	Aorta (?)				
Jansen (case 2) ^{9ff}	Aortic	Aneurysm at root of aorta				
Stæmmeler ^{9ll}	Mitral	Aorta				
Ku ^{9jj}	Pulmonary and tricuspid	Pulmonary artery, interven- tricular septum				
Norris U S Nav M Bull 30 37, 1932	Aortic (loss of commis- sure)	Aneurysm in sinus of Valsalva				
Case 1	Aortic	Interventricu- lar septum	Pulmonary	Interventricular septum		
Summary						
			Aortic	Pulmonary	Mitral	Tricuspid
Cases with actual invasion of the valves			8	5	2	3
Cases with juxtavalvular gummas, interfering with function of valves			4	8	0	2
Total cases with interference in valvular function			12	13	2	5

sion to the valvular or mural endocardium and pericardium may occur. Rarely, the heart valves (table), auricles,^{9t} epicardium or interventricular septum may be involved by direct extension from the roots of the aorta and pulmonary artery.

The two cases herein reported are examples of cardiac gummas differing markedly in the extent of damage produced. Case 1 is an instance of extensive gummatous aneurysm formation interfering with valvular function. On the other hand, case 2 was typical of the asymptomatic myocardial gumma. In neither case was the diagnosis made clinically. However, when atypical cardiac findings are observed in a patient with a condition known or suspected to be syphilis, tertiary cardiac syphilis (gumma) should be considered. The suspicion would be further enhanced in the presence of heart block, unusually situated weird stenotic murmurs and unexplained roentgen shadows at the borders of the heart. Positive serologic data may be lacking and are not essential for the diagnosis.

SUMMARY

Acquired tertiary syphilitic heart disease (exclusive of aortitis with commissural involvement) is uncommon and consists of circumscribed gummatous myocarditis (cardiac gumma) and diffuse gummatous myocarditis.

The authentic cases of cardiac gumma recorded in the literature have been enumerated, to these, two new cases are added.

Gummatous cardiac aneurysm generally occurs at the base of the left ventricle, where it is particularly apt to interfere with valvular function.

Involvement of the pericardium (especially of the visceral layer) occurs generally by extension from myocardial gummas and less often from gummatous lesions in the roots of the great arterial trunks.

Clinical recognition of the disease is rare. Unusually situated weird stenotic murmurs, unexplained roentgen shadows at the cardiac margins and heart block in a patient in whom syphilis is suspected suggest the possibility of tertiary cardiac syphilis, most likely gumma.

Laboratory Methods and Technical Notes

EMBEDDING IN GLYCOL STEARATE

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Orton and Post¹ in a search for an embedding material which would not interfere with the demonstration of fat in brain tissue tried di-glycol stearate. Having further tested this material as a general embedding agent, I have found a number of comparisons that may be made between it and paraffin, which it resembles in many respects.

Glycol stearate is a slightly yellowish, firm, waxy substance with a melting point near 50 C. It is water-diffusible. Tissue taken directly from an aqueous fixative and placed in the melted wax will become slowly impregnated by it. When melted, the wax is miscible in any proportion with 95 per cent alcohol. It is soluble in such substances as ether, chloroform, and xylene.

Blocks of tissue to be embedded are passed through graded percentages of alcohol as desired. They are then put into a mixture of equal parts of 95 per cent alcohol and glycol stearate and placed in an incubator at 56 C. for a period of from twelve to twenty-four hours. The tissues are then immersed in the pure melted wax and allowed to remain in the incubator for a period of time depending on the size of the blocks. If several blocks are being treated together, it is well to transfer them into fresh melted wax at least once during the process of impregnation. Blocks that are 2 mm. in thickness are thoroughly saturated by the wax at the end of twenty-four hours. If blocks are 4 mm. thick they should be left in the wax for forty-eight hours. Embedding is then performed in the same manner as when paraffin is used.

The time required for the process is greater than for some methods of embedding in paraffin, but the treatment with a mixture of alcohol and glycol stearate before the tissue is placed in pure wax materially hastens the process. In experimenting to find the proper length of time to leave the tissues in the melted wax, I have noticed that satisfactory sections can often be cut from blocks impregnated for only half the suggested time before embedding. When such blocks are examined a number of days or weeks after they have been embedded it is found that the tissues have shrunk away from the wax. This is obviously due to evaporation of the solvent remaining in the tissue and incomplete saturation of the tissue by glycol stearate.

When the blocks are removed from the molds after the wax has cooled and hardened, a little more care is required to avoid mutilation than when paraffin is used, for the stearate is more brittle than paraffin and adheres somewhat more persistently to the walls of the molds.

From the Pathology Department of the College of Medical Evangelists

1 Orton, S. T., and Post, J. Bull. Neurol. Inst. New York 2:302 (July) 1932

Sections are cut in the same manner as from paraffin blocks and nice ribbon sections can usually be made. As in the case of paraffin the sections sometimes roll up when cut. This is more liable to occur when the humidity or the temperature of the air in the room is low or when very thick sections are cut. As the melting point of glycol stearate is sharper than that of ordinary paraffin, the wax softens relatively little until it is heated to near this point. Therefore, in warm weather it is easier to cut sections from this material than from common paraffin.

Sections may be floated on the surface of warm water and then placed on slides or cover slips with a film of albumin fixative. The optimum temperature of the water to flatten the sections properly without melting the embedding material is 45 C.

The glycol stearate may be removed from the mounted sections by immersion for five minutes in chloroform or xylene, as in the case of paraffin-treated tissues. If all the wax does not appear to be dissolved at the end of five minutes, the quantity which remains usually will not interfere with staining. The alcohol baths commonly employed will aid in completing the removal of the glycol stearate.

Sometimes the sections fail to remain adherent to the slides when albumin fixative is employed. An alternative method of attaching sections to slides may be used with the aid of dropping bottles. Sections may be floated onto slides and then dried. The slides may be flooded with xylene for from two to five minutes, then blotted off and 95 per cent alcohol applied for one minute and blotted off. Thin pyroxylin may then be applied over the tissues as in the method frequently employed with frozen sections. Freshly embedded tissues always cut and mount better than those from older blocks.

The quality of the stained sections prepared by this method compares favorably with those embedded in paraffin. Cells show little distortion unless the blocks of tissue were not thoroughly impregnated and were allowed to dry out and shrink before being cut. Fats are too completely removed to be easily demonstrated in sections embedded by this method. The cost of the process is similar to that of embedding in paraffin.

General Review

WEIL'S DISEASE

REPORT OF A CASE WITH POSTMORTEM OBSERVATIONS AND
REVIEW OF RECENT LITERATURE

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In 1886 Weil¹ described a disease in which sudden onset, prostration, fever, muscular pain, jaundice, hemorrhagic tendencies and renal injury were the characteristic features. Inada² in 1916 reported that a spirochete was the causative agent. Noguchi³ classified this organism and named it *Leptospira icterohaemorrhagiae*. The disease is also called spirochetal jaundice or spirochaetosis icterohaemorrhagica.

Although many cases have been reported from Europe, Japan and the East Indies, this condition has apparently been extremely rare in the United States. Wadsworth⁴ in 1922 observed the first proved case in this country and called attention to the fact that the disease could be acquired from the accidental prick of a needle containing a virulent culture obtained from a common rat which harbored the spirochete. Ten other cases have been reported since that time from New York,⁵ Virginia,⁶ District of Columbia,⁷ Pennsylvania⁸ and California.⁹ No case has previously been recorded from New England.

From the Fifth Medical Service, Boston City Hospital, the Department of Medicine, Boston University Medical School, the Mallory Institute of Pathology, Boston City Hospital, and the Evans Memorial.

1 Weil, H A. *Deutsches Arch f klin Med* **39** 209, 1886

2 Inada, R, Ido, Y, Hoki, R, Kaneko, R, and Ito, H. *J Exper Med* **23** 377, 1916

3 Noguchi, H. *J Exper Med* **27** 575, 1918

4 Wadsworth, A, Langworthy, V, Stewart, C, Moore, A, and Coleman, M. *J A M A* **78** 1120, 1922

5 (a) McDowell, E S. *New York State J Med* **25** 19, 1925. (b) Cushing, E H. *J A M A* **89** 1014, 1927

6 Mulholland, A B, and Bray, W E. *J A M A* **90** 1113, 1928

7 Towler, H H, and Walker, J E. *J A M A* **89** 86, 1927

8 (a) Sailer, J. *Am J M Sc* **170** 332, 1925. (b) Hayman, J M, and Lynch, F B. *ibid* **173** 8, 1927

9 Ball, A H. *Am J Clin Path* **3** 283, 1933

It is our purpose in this paper to add the twelfth proved case, to review the pathologic and clinical aspects of the disease, to discuss all the reported American cases and to point out certain facts derived from a study of the recent literature which will aid in the understanding of this disease entity

The paucity of cases recorded in the United States may be due either to the rarity of the disease or to the fact that the clinical picture and the proper laboratory methods of diagnosis are not generally known. That the latter explanation seems more reasonable was well brought out by the extensive work of Schuffner¹⁰ in the Netherlands. By means of a perfected agglutination test he was able to diagnose many cases not recognized clinically. Fairley,¹¹ having found 1 typical case in a sewer worker in London, applied the same agglutination test to all sewer workers who gave a past history of any illness resembling Weil's disease, and he discovered 7 instances in which the disease had not originally been diagnosed. Davidson,¹² in Scotland using a similar method, was able to collect 23 cases among fish cutters, whose trade had not previously been suspected of exposing one to the organism. These and similar studies have resulted in a sharp increase in the number of cases reported from England, the Netherlands and other countries.

It has been known since the World War¹³ that jaundice is not constantly present in Weil's disease. In the literature since that time this point has either not been mentioned or considered as a clinical oddity. However, a study of cases of the disease in the Netherlands, England and France in recent years in which Schuffner's diagnostic procedures were used revealed the significant fact that over 50 per cent of patients with Weil's disease do not have jaundice. In consequence it has become the practice of European workers to refer to the condition as Weil's disease in preference to those synonyms which emphasize "jaundice." A similar nomenclature should be adopted in this country.

REPORT OF CASE

History and Course—The patient, a 38 year old white man (a fish cutter), entered the Fifth Medical Service of the Boston City Hospital at 5 p. m. on May 31, 1934, with the complaint of severe prostration and jaundice. The history was secured from his family, as the patient was too ill to answer rationally.

The patient was in excellent health until seven days before admission to the hospital, when he noticed lassitude, anorexia and progressive pains and weakness in the muscles of both legs. At about the same time rigor of moderate severity developed followed by high fever. By the end of the first day he was forced to

10 Schuffner, W. Tr. Roy. Soc. Trop. Med. & Hyg. **28** 7, 1934.

11 Fairley, N. H. Brit. M. J. **2** 10, 1934.

12 Davidson, L. S., Campbell, M. A., Rae, H. J., and Smith, J. Brit. M. J. **2** 3859, 1934.

13 (a) Valassopoulos, A. Bull. et mem. Soc. med. d. hop. de Paris **41** 920, 1917. (b) Dawson, B., Hume, W., and Bedson, S. Brit. M. J. **2** 345, 1917. (c) Stokes, A., Ryle, J. A., and Tytler, W. H. Lancet **1** 142, 1917.

remain in bed. He continued in this condition for four days, when it was noticed that his skin was "yellow" and the urine dark red. No attention was given to the character of the stools. After the onset of the jaundice there were nausea and vomiting but no abdominal pain. Severe hiccups developed and persisted for three days. The family noticed that during all this time he appeared unusually weak and was unable to attend to even minor personal duties. The patient continued to fail rapidly and was sent to the hospital on the eighth day of his illness.

The family and the social history, except the patient's trade, were of no significance.

The family stated that the patient had never been sick since childhood and had not missed a day of work for over a year. He had never received antisyphilitic therapy or other medication. There was no history of the use of alcohol.

On admission the temperature was 99 F, the pulse rate 100 per minute and the respiratory rate 34. Examination revealed a markedly jaundiced man with dull senses, who was severely prostrated and able to utter only a few unintelligent words. He hiccuped persistently. There was no stiffness of the neck. The sclerae and skin were intensely icteric and the conjunctivae moderately injected. The pupils were small and regular in shape and reacted sluggishly to light. A slight herpetic eruption was present on the lips. The tongue was dry and cracked, and there was much dried blood on the dorsum and about the lips. The gingival margins were soft and bled on slight trauma. No injection of the blood vessels of the pharynx was noticed. There was no evident adenopathy. Auscultation of the chest revealed nothing abnormal except a few scattered moist râles. The heart sounds were rapid and of poor quality, but of regular rhythm. On percussion the borders of the heart seemed within normal limits. The blood pressure was 120 mm of mercury systolic and 50 mm diastolic. The pulse was regular but of poor volume. The abdomen was distended. On percussion the liver seemed enlarged, but owing to the distention it could not be satisfactorily palpated. The spleen was not palpable. The genitalia were normal. Swelling and tenderness were marked in the muscles of the right thigh and less evident in other groups of muscles. The tendon reflexes were present but sluggish. No pathologic reflexes were elicited. An examination of the rectum gave negative results.

The urine was concentrated and bile-stained and contained albumin and granular casts. The white blood cell count was 28,750 per cubic millimeter, while the red cell count was 3,130,000. The hemoglobin reading was 68 per cent on the Sahli scale. A stained blood smear revealed a differential count of 95 per cent polymorphonuclear leukocytes and 5 per cent lymphocytes.

The report of the chemical analysis of the blood was as follows: nonprotein nitrogen, 200 mg per hundred cubic centimeters; sugar, 55 mg; total protein, 4.4 Gm, with an albumin-globulin ratio of 31.69; cholesterol, 90 mg; calcium, 10 mg; and phosphorus, 13.6 mg. One culture of the blood resulted in a growth of *Staphylococcus aureus*. The stool was liquid and pale tan and gave a strongly positive reaction to the benzidine test for occult blood. The Wassermann reaction was negative on two occasions.

The patient lapsed into coma shortly after admission. He received 1,500 cc of a physiologic solution of sodium chloride by hypodermoclysis, 100 cc of a 50 per cent solution of dextrose and an ampule of calcium gluconate intravenously, caffeine and an enema.

The respirations became of the Cheyne-Stokes type and the pulse thready. The chest began to show many scattered moist râles, and the pupils were dilated. The temperature rose to 100.5 F. Death occurred at 9 a. m. on June 1, 1934, after sixteen hours of hospitalization. An autopsy was performed.

Macroscopic Examination—The body was that of a well developed, well nourished white man with generalized jaundice (the skin was "bright orange-yellow")

Peritoneal Cavity About 20 cc of clear orange-brown fluid was present

Pleural Cavity A few fibrous adhesions bound the upper lobe of the left lung to the anterolateral portion of the parietal pleura

Heart The heart weighed 340 Gm and appeared normal

Lungs The lungs weighed 750 and 730 Gm, respectively, and were congested and edematous, yielding a thin, pale red fluid on pressure The trachea and bronchi were normal

Spleen The spleen weighed 120 Gm and was somewhat soft

Liver The liver weighed 2,100 Gm and was smooth, greenish brown and of average firmness The cut surface was brownish gray The gallbladder and bile ducts were normal

Kidneys The kidneys weighed 320 and 210 Gm, respectively The capsule stripped easily from a smooth surface devoid of hemorrhages The cortex was 8 mm in thickness and was greenish brown throughout The pyramids were congested and the pelves normal

Stomach Marked generalized congestion of the gastric mucosa was present

Ileum Small hemorrhagic areas were noted in the mucosa of the lower part of the ileum

Esophagus The mucosa of the greater portion was dark red and soft and presented a "chewed-up" appearance There was a slightly greenish cast in places

Gastrocnemius Muscle The muscle was a uniform red

Aorta Slight atheroma was noted

Brain The weight was 1,460 Gm The pia-arachnoid was faintly yellow

The adrenals, pancreas, bladder, ureters, genital organs and vertebral marrow were normal

A culture of blood from the heart produced no growth No evidence of septicemia was found The antemortem finding of *Staphylococcus aureus* was attributed to contamination

Microscopic Examination—*Heart* Rarely, a single muscle fiber was vacuolated or broken up into longitudinal hyaline strands with loss of striations and was strikingly infiltrated with mononuclear cells (histiocytes) and leukocytes An occasional group of plasma cells adjoining a venule was noted Occasional slight scarring, chiefly in the neighboring vessels, was present

Lung Small patches of fresh hemorrhage into the alveoli were noted The walls were crowded with polymorphonuclear leukocytes, which were present in moderate numbers in the bronchial walls Mononuclears containing carbon were fairly numerous

Spleen No hemosiderin or appreciable phagocytosis of red cells was apparent There was increase of the plasma cells and, to a slight degree, of the polymorphonuclear leukocytes Small deposits of neutral fat were noted in the reticular cells of the malpighian corpuscles

Liver There was no biliary stasis Occasional small droplets of bile were observed in cells in the center of the lobule The nuclei were often swollen Binucleate cells were common Many mitoses, multiple in a few instances, were noted There was dense lymphocytic infiltration of some of the portal spaces without relation to the small bile ducts The Kupffer cells often contained bile pigment and occasionally a small droplet of fat Practically no fat was present in the hepatic cells The perisinusoidal lymph spaces were usually wide and clearly defined

Pancreas Inspissated secretion and occasional polymorphonuclear leukocytes were observed in the dilated acini in scattered areas. Necrosis occurred in the center of one lobule, with sparse infiltration of the stroma by polymorphonuclear leukocytes.

Kidney Marked interstitial infiltration, for the most part in the medulla, both diffuse and in foci, was chiefly of plasma cells, with occasional eosinophils, polymorphonuclear leukocytes or mononuclear cells. Two well developed follicles of small lymphocytes were noted. The tubules in this region contained considerable cellular debris and red granular material and occasionally a few polymorphonuclear leukocytes or mononuclear cells filled with dark bile pigment. Some tubules were much dilated, with evidence of regeneration of epithelium. Perhaps a fourth of the convoluted tubules showed swelling of the epithelium with narrowing of the lumen. A negligible amount of fat was present, but only in extremely finely divided form. The glomeruli were normal.

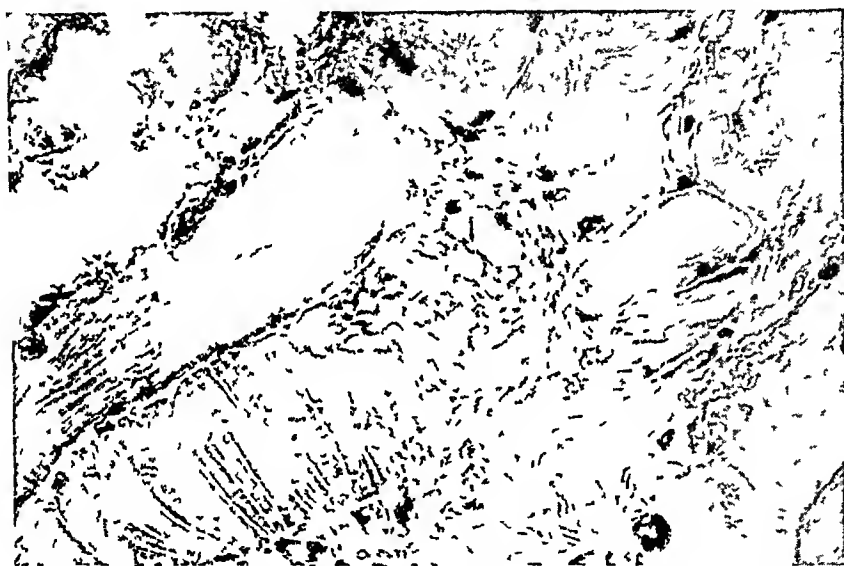


Fig 1—Photomicrograph of gastrocnemius muscle, showing the characteristic selective involvement of fibers and the marked vacuolation of one

Adrenals The cortical cells appeared depleted of lipid droplets, but a stain for fat showed a fair amount present in finely divided form.

Vertebral Marrow The marrow of the vertebrae was normal. No hemosiderin or increased phagocytosis of the red cells was noted. Megakaryocytes were numerous.

Esophagus A massive exudate of blood, polymorphonuclear leukocytes and fibrin into and beneath the squamous epithelium was observed. In places the cells were lifted up and teased apart as though by fluid. Masses of cocci and other organisms occurred at the surface. The submucosa was edematous, the outer part was infiltrated and the vessels were crowded with polymorphonuclear cells. Several large isolated foci of lymphocytes were noted in the deeper portion.

Gastrocnemius Muscle (fig 1) Necrosis of one or two or three adjoining fibers and local accumulation of mononuclear and plasma cells and occasional polymorphonuclear leukocytes were noted. The degenerated fiber was broken up into a few large, round hyaline masses, with slightly basophilic multinucleate remnants.

of sarcolemma, often triangular, between them or at one side. Marked vacuolation of fibers at the margin of the area of degeneration was present.

Stomach, Brain and Choroid Plexus. The results of examination were negative. Levaditi stains were made of tissue from the kidney, liver, spleen, gastrocnemius muscle and lung. Spirochetes were seen only in the convoluted tubules of the kidney. From about six to a dozen organisms were present in a tubule, but only in three or four tubules in a section. A few appeared to be just within the cytoplasm of the epithelial cells, but there was no corresponding local reaction or necrosis (fig. 2).

A few suggestive forms were present in the muscle, occurring singly in the stroma or as groups of irregular rods (as though fragmented) within degenerating fibers. None were in or near the vacuoles.

Diagnosis. The final diagnosis in this case was established post mortem by the characteristic appearance of the liver, kidney and gastrocnemius muscle and



Fig. 2—Spirochete in a tubule of the kidney. Two others lie at the left, apparently within the epithelium. Levaditi stain, oil immersion lens.

by demonstration of the spirochetes in the kidney. Inoculation of a guinea-pig and culture of the organisms unfortunately were not done.

Epidemiological Survey.—The patient was employed as fish cutter at a local plant known to be infested with rats. The fish (chiefly haddock and small cod) were brought in and the refuse removed on wooden conveyers. The cutting and filleting were done at fairly clean, but necessarily slimy, wooden benches. The floor of concrete was poorly drained and covered with dirty water and slime. The men wore rubber boots and gloves but were subject to cracks and slight cuts on the hands. The room was cleaned regularly once a week.

No cases of a similar nature could be recalled by any of the employees interviewed, and the physician who had charge of patients from this company and similar companies, as well as of many sewer workers and longshoremen, had no record of a similar case. Since this company doctor cared only for traumatic disabilities his statement meant little. Systemic disorders were treated by the personal physician of the employee. The living premises of the patient were not unusual. No other cases were reported either in the family or in the neighborhood.

The following procedures are advised in autopsies in suspected cases

1 Examination by dark field of fresh scrapings from the kidney

2 Histologic examination of (*a*) the skin (to check further the observations of Pick¹⁴), (*b*) the diaphragm, in cases in which hiccup was prominent, (*c*) the muscles of the eye if pain behind the eyes was a prominent feature (*d*) the tonsils, pharynx, esophagus or other possible points of entry showing signs of inflammation and (*e*) lymph nodes in the corresponding region which are enlarged

3 Fixation of all tissues to be examined for spirochetes in a solution of formaldehyde which is the proper fixative when the Levaditi stain is to be made

THE INFECTING ORGANISM

Leptospira icterohaemorrhagiae is a true spirochete, with a central axial filament¹⁵ It is from 6 to 15 microns long and approximately 0.2 micron wide, tending to be smaller in human subjects than in guinea-pigs Fine, tightly wound spirals enclose the axial filament in the stiffer middle part and are prolonged beyond it into straight, curved or hook-shaped ends, which are of greater mobility

The organism is rapidly motile and moves in a straight line by spinning vigorously on its axis, one end being curved and perhaps acting as a propeller while the middle part and the other end remain straight Motion is in the direction of the straight end Division takes place by longitudinal fission It differs from the treponemes in being insoluble in a 10 per cent solution of saponin¹⁶ Specific anti-serum or a solution of sodium taurocholate produces lysis progressing from the ends toward the middle portion

EPIDEMIOLOGY

Nonpathogenic saprophytic spirochetes morphologically similar to that of Weil's disease occur in natural waters all over the world They were first described in 1914 by Wolbach and Binger,¹⁷ who discovered them in stagnant water obtained from a pond near Boston They have since been found in mud of lakes and rivers, in garden soil, among decayed leaves, in puddles, surface-water and ditch-water, in sewers and reservoirs, in salt springs and sea water and in the tap water of many European cities,¹⁵ as well as in 87.5 per cent of samples of municipal

14 Pick, L. *Berl klin Wchnschr* **54** 451 and 481, 1917

15 Uhlenhuth, P., and Fromme, W. *Weilsche Krankheit*, in Kolle, W., Kraus, R., and Uhlenhuth, P. *Handbuch der pathogenen Mikroorganismen*, Jena, Gustav Fischer, 1930, vol 7, pt 1, p 487

16 Noguchi, H. *J Exper Med* **27** 609, 1918

17 Wolbach, S., and Binger, L. *J M Research* **30** 23, 1914

drinking water from forty-seven cities in the New England area¹⁸ These water spirochetes have all come to be referred to under the common specific name *Leptospira biflexa* Actually, the group is composed of a great number of strains of close serologic relationship¹⁹ In addition to *Leptospira* (properly *Spirochaeta*) *icterohaemorrhagiae* of Weil's disease pathogenic strains include (1) *Leptospira hebdomadis*, the causative organism of akiyami or seven day fever in Japan, (2) *Leptospira grippo-typhosa* which causes the swamp fever of eastern Europe, and (3) *Leptospira canicola* which produces a disorder in dogs resembling Weil's disease

Weil's disease itself has been reported from all over the world, and Schuffner determined the approximate serologic identity of original strains from the Netherlands, Berlin, London, Paris, Japan, the East Indies, Lisbon Hamburg and New York (the strains of Noguchi) Serums from Greece, Copenhagen and the Belgian Congo also corresponded, an observation leading to the conclusion that a universal specificity exists¹⁰

The virulent Weil strain has never been found except in places accessible to or infested by wild rats These animals carry the organisms in the tubules of the kidney excreting them in the urine and thus inoculating water, soil or food with which they come in contact Rats from all over the world have been examined, and the spirochete was found to be present in roughly 10 per cent

The following percentages for frequency of infestation among the rats examined in American cities were determined Chicago, 3, Baltimore, 7 and 52, Washington, 10, Nashville, 10, and New York 17 and 22¹⁵ In the Netherlands 45 per cent of adult rats but only 3 per cent of young ones, carried the spirochete¹⁰ Both Norwegian and black rats, as well as mice, are implicated They presumably pick up the spirochete by eating infected material Feeding experiments have shown in a few instances that they can be infected by this route Rat-to-rat infection may occur in cages²⁰ Men have been infected by rat bite in at least 2 instances²¹

Dogs may act as vectors, since they are subject to infection by the true Weil strain as well as by *L. canicola*¹⁰ No case of human infection from a dog due to a human strain has been reported However, Schuffner was able to isolate *L. canicola* from the blood of a man suffering from an infection resembling Weil's disease, and he definitely proved that this strain could produce in man a disease simulating true

18 Dimitroff, V T J Infect Dis 40 508, 1927

19 Uhlenhuth, P, and Zuelzer, M Klin Wchnschr 1 2124, 1922 Baermann, G, and Zuelzer, M Zentralbl f Bakt (Abt 1) 105 345, 1928

20 Uhlenhuth, P Munchen med Wchnschr 77 2047 and 2098, 1930

21 Ido, Y, Hoki, R, Ito, H, and Wam, H J Exper Med 26 341, 1917

Weil's disease¹⁰ Certain flies (*Haematopota* and *Stomoxys*), lice and horse-leeches have been shown capable of transmitting the infection experimentally but are thought to be of no importance under natural conditions¹⁵ Nonvirulent spirochetes were found in the urine of 2 persons exposed to patients with Weil's disease and their excreta,²² but there is little evidence that man may act as a carrier

Dissemination by water occurred in a unique epidemic in Lisbon in 1931²³ Drinking water obtained at a public fountain was responsible Rat feces were found in the underground aqueduct

Among the places in which human beings have contracted the disease are damp mines, rice-fields flooded with fecally contaminated water, sewers, canals, drainage ditches, stagnant moats, trenches (in war time), garbage pits, piggeries, breweries, slaughter-houses, moist soil or water containing decaying matter and public baths, where the hazard from rats is great and the water but slowly changed Alkalinity of the soil or water is favorable to the organisms, salinity is unfavorable¹⁰

Whether or not virulent leptospirosis may occur in regions free from rats (if such places exist) remains to be determined Buchanan²⁴ recovered them from the roof slime of a mine in a situation which appeared inaccessible to rats, but other parts of the mine were infested Whether or not the common saprophytic form can undergo mutation or acquire increased virulence in the presence of abundant decaying matter or in the animal kidney is a question Such an apparent transformation of a nonpathogenic strain into a virulent form took place during prolonged cultivation on serum medium and, in another instance, by repeated passage through guinea-pigs²⁰ For all purposes, however, one may say No rats, no virulence

Incidence—Extensive epidemics of jaundice occurred among Napoleon's soldiers in Egypt, during the American Civil War and in the Boer war It is not determinable whether these cases represent instances of infectious jaundice or instances of Weil's disease After the discovery of the causative organism, verified cases of Weil's disease were reported during the World War on the Italian front and in the British, German and French armies

In recent years its occurrence has been chiefly among (1) persons working in infested places as previously enumerated, i e., miners, sewer workers, slaughter-house workers, butchers, sluice or drain cleaners, barge-men and fishermen, (2) persons who have fallen into polluted waters accidentally or with suicidal intent and (3) bathers and swim-

²² Frugoni, C, and Capellani, S *Riforma med* **33** 439, 1917, quoted by Uhlenhuth and Fromme¹⁵

²³ Jorge, R *Bull Office internat d'hyg pub* **24** 88, 1932

²⁴ Buchanan, G *Spirochaetal Jaundice*, Medical Research Council, Spec Rep Ser no 113, London, His Majesty's Stationery Office, 1927

mers Between 1924 and 1933 in the Netherlands there were 452 cases, the incidence varying from 22 to 56 for every 400,000 in the three more densely populated coastal provinces¹⁰ In the city of Dordrecht 30 cases were traced to occupational circumstances, 44 to "water accidents" and 128 to bathing and swimming The incidence of cases due to water accidents (1 case for every 75 accidents) was notably greater than the highest figure found for any bathing place (about 1 for every 4,200 baths) The greatest prevalence of infection was in the late summer (from July to October), but cases occurred in all months of the year Statistics for other countries, where laboratory tests for aid in diagnosis were commonly made, are similar

Port of Entry of Organism—Guinea-pigs have been infected by rubbing the spirochetes into the skin² Pigs with slightly scarified skin acquired the disease on being made to swim in water from heavily infected ditches²⁵ Definite infection through cuts and needle pricks has been occasionally reported²⁶ It is probable that a considerable number of human infections through the skin have occurred among barefooted workers in rice-fields, bathers, butchers and sewer workers with cracks, cuts or scratches on the feet, hands or elsewhere Local enlargement of the lymph nodes in certain cases favors this view²⁷

Infection per os has been accomplished experimentally by a few investigators in both rats and guinea-pigs, although most attempts have been unsuccessful The acid of the stomach and the bile in the duodenum exert a lethal effect on the organisms,¹⁵ and the buccal and the pharyngeal mucosa appear a more probable port of entry than the gastro-intestinal tract, although Inada favored the latter route The greater frequency among victims of water accidents as compared with that among bathers has been attributed to unintentional swallowing or inhaling of water by the former persons¹⁰ The epidemic in Lisbon arising from drinking water is the outstanding example of infection by the oral route,²³ and certainly in the majority of situations conveyance of infected material to the mouth by the hands would be difficult to rule out

Accidental laboratory infection occurred in 2 workers who were sprayed in the eyes, 1 with highly infected guinea-pig blood²⁸ and the other with a pure culture of a virulent strain of *Leptospira* Marked injection of the conjunctival vessels is a regular and early symptom

25 Appelman, J. M. Het isoleeren van *Leptospira icterohaemorrhagiae* uit water, Leyden, N. V. Leidsche Drukkerij, Morschlingel, 1934, abstr., Trop. Dis. Bull. 31: 514, 1934

26 Wadsworth and associates⁴ Schuffner¹⁰

27 Drew, J. G. Brit. M. J. 2: 1142, 1934 Inada and others²

28 Goebel, W. Med. Klin. 12: 381, 1916

in both men and animals, but it has nothing to do with the primary site of infection

There has been 1 case of alleged transmission from husband to wife by cohabitation²⁹

Excretion—In the guinea-pig spirochetes are excreted in large numbers in the urine, bile and bronchial secretions³⁰ In human patients they are excreted in the urine, they have also been reported in the feces² and in isolated instances in the sputum² and vomitus³¹ Their presence in blood from menstruation or epistaxis is to be expected Supposed infection from the urine has been observed in only 1 instance¹⁵

Period of Incubation—According to Inada, the period of incubation is from five to seven days In 37 cases of infection due to water accidents in which the interval was accurately fixed Schuffner found it to vary between four and nineteen days, usually from seven to thirteen days, with an average of 10.3 days¹⁰

Prevention—Obvious measures include elimination of rats, removal of decaying matter and sources of pollution of water and chlorination of the water supply Inada³² was able to reduce substantially the incidence in the coal mines by pumping out the water He recommended drainage and application of lime to infected soil Eminently successful results in the rice-fields were obtained by Tohyama³¹ He found calcium cyanamide to be the only substance which would kill the spirochete and at the same time fertilize the rice, he determined that application of the equivalent of 4.17 pounds (1,891.5 Gm) per acre (4,046.87 square meters) provided a sufficient concentration to accomplish the former result

Persons coming into contact with patients should be advised of the possibility of contagion All dejecta must be carefully removed and destroyed The urine of a convalescent patient may be examined after forty days to determine whether he has become a carrier

Prophylaxis—Active immunization by means of vaccination has strikingly lowered the incidence in many instances In certain regions of Japan there were but 5 cases per month where there had been from 15 to 30 before³² Wani vaccinated 10,368 persons in six coal mines, with a resulting morbidity rate of 0.12 per cent, or less than one-ninth that among unvaccinated persons in another mine³³ Baermann found no cases among 80 vaccinated men, although they continued

29 Doeleman, quoted by Schuffner¹⁰

30 Clement, P, and Fiessinger, N Bull et mem Soc méd d hop de Paris
40 2073, 1916

31 Tohyama, Y Japan M World 4 193, 1924

32 Inada, R Japan M World 2 189, 1922

33 Wani, H, quoted by Inada³²

to work in an infected district³⁴ According to Noguchi, the vaccine to be effective should contain 200,000,000 organisms per cubic centimeter Inada used organisms grown on Noguchi's medium and was able to immunize guinea-pigs with 0.01 cc Baermann used a mixture of about 116 strains, the organisms were killed by heating to 65 C and a 0.5 per cent solution of phenol was added¹⁵ Wani gave doses of 2 and of 3 cc subcutaneously one week apart He was able to demonstrate spirocheticidal properties of the blood as late as nineteen months after vaccination Baermann used a single dose of 5 cc intramuscularly or doses of 1 and 2 cc from five to eight days apart³⁵

Because of the rarity of the disease in this country active immunization on a large scale would not be practicable It is strongly advised, however, for persons in certain occupations, including sewer workers and bacteriologists known to be exposed to infection

Immunity—Although hens and frogs possess antispiochetal substances in their blood, there is no natural immunity in man¹⁵ A powerful active immunity, however, is acquired by an attack of the disease Only 1 case of a second attack in the same person has ever been reported^{35a} This immunity is at least partly due to antibodies which appear in the blood between the seventh and the fifteenth day of illness³⁶ They attain their highest concentration during convalescence and may persist for many years Postmus³⁷ found these antibodies well developed even after eight years or longer In 1 person they were present after twenty-two years¹⁵ In a patient receiving serum during an attack the resulting immunity may be lower than that of untreated patients Schuffner cited the case of a treated patient the titer of whose serum was 1:3,000, whereas without serum treatment the titer is commonly 1:30,000

Passive immunity is successfully conferred by administration of either convalescent serum or immune horse serum Inada and his associates³⁶ were able to protect guinea-pigs with serum from convalescent patients or from immunized goats³⁸ They also immunized horses, and with antiserum given to patients admitted before the seventh day of the disease they were able to reduce the mortality rate in their severest cases from 57.1 to 38.5 per cent Of 21 patients under the

34 Baermann, G, and Smits, E. *Zentralbl f Bakt (Abt 1)* **105** 368, 1928

35 (a) Baermann, G. *Die kurzfristigen Spirochätenfieber*, in Kolle, W, Kraus, R, and Uhlenhuth, P. *Handbuch der pathogenen Mikroorganismen* Jena, Gustav Fischer, 1930, vol 7, pt 1, p 661 (b) Uhlenhuth and Fromme¹⁵

36 Inada, R, Ido, Y, Hoki, R, Ito, H, and Wani, H. *J Exper Med* **24** 485, 1916

37 Postmus, S. *Nederl tijdschr v geneesk* **4** 2648, 1933

38 Donkeys, sheep or rabbits are also suitable

age of 40 only 1 died. A rapid disappearance of spirochetes from the blood was observed.

MORBID ANATOMY

The clinical effects of this disease are those of a severe generalized infection of the blood stream. The early extreme prostration is perhaps the most striking manifestation. Accompanying organic changes are somewhat variable and may be relatively slight.

The usual observations at autopsy are generalized jaundice and lesions characteristically involving the following structures: (1) capillaries, (2) liver, (3) kidneys and (4) skeletal muscle.

1. Damage to the capillaries is manifested by minute hemorrhages generally distributed in the body. They are most common under the peritoneum and pleura and in the gastro-intestinal tract, kidneys, adrenals, nasal mucosa and skin. They may also occur beneath the endocardium or pericardium, under the capsule or within the portal spaces of the liver, in the mesentery, spleen or pancreas, beneath the mucosa of the larynx and trachea, in the lungs and in the mucosa of the bladder.

The brain may contain pea-sized hemorrhages³⁹ or the tentorium and dura may be involved.⁴⁰ Miller noted hemorrhages in the tibial nerve.³⁹

These hemorrhages are considered to be the result of a local toxic effect of the spirochete on the wall of the vessel. Diapedesis of red cells through the unbroken wall was observed in the lung by Beitzke.⁴⁰ Hemorrhages are of considerable importance in the severer cases, accounting for the epistaxis, hematuria, hematemesis, melena, hemoptysis or purpura which is sometimes present. Death may even result from gastro-intestinal hemorrhage.¹⁴

2. The liver may appear normal on gross examination. It is commonly slightly enlarged and often bile-stained,⁴¹ never shrunken as in acute yellow atrophy. Obstruction of intrahepatic or extrahepatic bile ducts is lacking. (Plugs of mucus and epithelium in the lower end of the common duct have been described only in rare instances.⁴²)

Microscopically, two significant changes occur with fair regularity: proliferation of hepatic cells and traces of biliary stasis in the central part of the lobule. The hepatic cells show active division, which may be predominantly amitotic.⁴³ The nuclei are often swollen, and there

39 Miller, J. W. *München med Wchnschr* **64** 1572, 1917.

40 Beitzke, H. *Berl klin Wchnschr* **53** 188, 1916.

41 Garner, M., and Reilly, J. *Arch de méd exper et d'anat path* **28** 228, 1918.

42 Oberndorfer, J. *München med Wchnschr* **65** 1190, 1918.

43 Verne, J., Barietv, M., and Albeaux-Fernet, M. *Ann d'anat path* **9** 200, 1932.

are sometimes two or three in a cell. Mitoses may be frequent, however. Cloudy swelling, loosening or dissociation of the cells as though from edema and widening of the perisinusoidal lymph spaces have been described.^{13b} Fatty infiltration is absent or slight. Retained bile usually occurs in small amount centrally in the lobule within a few bile capillaries or as small droplets or granules in the cytoplasm of the cells, there is none in the periphery of the lobule nor is there evidence of stasis in the interlobular or larger bile ducts.

Other changes include swelling and homogeneization of the chondriome,⁴³ deposition of fat in the Kupfer cells, infiltration of the portal spaces by lymphocytes and a few polymorphonuclear leukocytes or eosinophils.

In some cases the liver has shown varying degrees of necrosis, usually slight and focal but occasionally so extensive as to simulate that of acute yellow atrophy. There is no marked reduction in size, however. In 1 patient, dying on the twelfth day, the liver was grayish brown, with the centers of the lobules dark red, as seen on the cut surface. It weighed 1,475 Gm.⁴⁴ The diagnosis was confirmed by concomitant myositis. Such conditions are regarded simply as the result of an unusually severe infection.

How will one account for the jaundice? There are two explanations: (a) that swelling of the cells and edema (Beitzke's "toxic edema") produce mechanical obstruction by compression of the bile capillaries and (b) that the hepatic cells are so damaged by the spirochetal toxin that their bile-excreting function is crippled. Probably both factors are involved. Their relative importance could be better estimated if tissue could be studied from the earliest days of the disease. At all events, the stimulus which produced the rather uniform, diffuse regeneration-like appearance in the later stage may conceivably have arisen from an early generalized toxic influence insufficient to cause actual necrosis. Excessive hemolysis is apparently not a feature. Increased phagocytosis of red cells in the reticulo-endothelial system is only inconstantly observed.⁴⁰ Fragility of the red cells is not increased.^{13b}

3. The kidneys are often enlarged and usually show (a) the greenish-brown stain common to bile-saturated organs in any case of jaundice (b) swelling and more or less marked necrosis of the epithelium of the convoluted tubules and (c) interstitial infiltration consisting chiefly of lymphocytes, with smaller numbers of polymorphonuclear leukocytes and eosinophils.⁴⁰ There may be small hemorrhages under the capsule, into the interstitial tissue or into the tubules. Granular and bile-containing casts are common. Little fat is present. The glomeruli are unchanged.

⁴⁴ Bates, J. E. *Canad. M. A. J.* **16** 1466, 1926.

in most cases. The damage is thus predominantly in the tubules. According to Pick, it is toxic in origin and due to the spirochetes, not to the bile.

Other changes sometimes observed include swelling or proliferation of the epithelium of Bowman's capsule, crescent formation, acute hemorrhagic glomerulonephritis,^{5a} acute nephritis resembling that of scarlet fever,¹⁴ marked interstitial infiltration by polymorphonuclear leukocytes almost to the extent of abscess formation⁴⁵ and infiltration by great numbers of eosinophils.

The extent of the renal injury is often better indicated by the clinical and laboratory findings than by the histologic structure, as in the case reported in this paper.

4. The muscles most severely involved are those of the calf. Others, including the pectoral muscles, are similarly but less affected. The gross appearance is usually normal,¹⁴ but there may be punctate hemorrhages or bile-stained foci of degeneration as large as from 4 to 5 mm in diameter.

As seen microscopically the process characteristically selects isolated fibers and only part of the fiber, with more extensive involvement adjacent fibers or even a whole field are concerned. There are vacuolation, swelling, loss of striations, hyalinization, infiltration with histiocytes, polymorphonuclear leukocytes and plasma cells, breaking up of the substance of the fiber into large, round lumps of hyaline material, resorption and proliferation of the nuclei of the sarcolemma. Hemorrhage into the empty sheath has been observed.

The picture differs strongly from that of Zenker's degeneration, such as occurs in typhoid fever. The latter form may usually be detected macroscopically, involves chiefly the adductor and abdominal muscles, commonly gives rise to hematoma, affects all the fibers in large areas and causes swelling of the fibers without resorption and shrinkage.

The lungs are sometimes normal. Congestion and edema may be marked. The pleural surface may be strikingly spotted with hemorrhages.¹⁴ Confluent patches of hemorrhage in the parenchyma resemble infarcts.⁴⁵ There may be miliary pneumonic foci.¹⁵ The accompanying bronchial secretion is sanguinopurulent.³⁹ The gross appearance of the lung is more characteristic in the guinea-pig.

The heart may show scanty cellular infiltration around the small vessels⁴⁰ or in the myocardial stroma. In 1 case there were large areas of degeneration of the muscle fibers of the type seen in skeletal muscle, i. e., vacuolation, breaking up into lumps and proliferation of the sarcolemma.⁴⁶ Fibrinous pericarditis was noted in 1 instance by Pick.

45 Hart, C. *Berl klin Wchnschr* 54 285, 1917.

46 Reinhardt. *Med Klin* 13 981, 1917, abstr, *Munchen med Wchnschr* 64 1403, 1917.

Miller³⁹ observed a large area of infarct-like necrosis in the wall of the left ventricle with hemorrhage and local pericarditis. Dragert⁴⁷ reported 2 cases of vegetative endocarditis and demonstrated the spirochete in the vegetation, which was on the interventricular septum 2 cm below the aortic valve in 1 case and at one of the commissures of the valve in the other.

The spleen is usually not enlarged and is of moderately firm consistency. Hemorrhages, deposits of hemosiderin and numbers of phagocytes with ingested red cells are occasionally seen.²¹

The gastric mucosa is commonly diffusely peppered with petechial spots, serious hemorrhage may arise in this area or from points in the small or the large bowel. The duodenal mucosa may be swollen and bluish red^{13c} and the wall hemorrhagic.¹⁸ Catarrhal inflammation may occur in the duodenum and terminal portion of the ileum.⁴⁹ Petechial spots under the serosa are common throughout the tract. In 1 case of colitis, foci of hemorrhage and necrosis⁴⁷ were described. How great a part is played by uremia in the production of these lesions has not been determined.

The pancreas was hemorrhagic throughout in 2 of Mayer's cases, hemorrhages into the wall of the duodenum and the ampulla also were present.⁴⁸ Flabby consistency of the organ and small size of the cells were noted,⁴⁰ marked edema was noted in 1 case and foci of necrobiosis in another.³⁹

The adrenals fairly constantly show hemorrhage. In 1 case the whole organ was involved, the hemorrhage apparently starting in the medulla.^{13b} In another case the picture of total hemorrhagic infarction was presented.⁴⁸

Lymph nodes were occasionally seen to be enlarged in the upper part of the cervical region only, by several investigators.⁵⁰ Swelling of local nodes was noted by Japanese investigators in cases of infection supposedly through the skin.²⁷

In the tonsils Miller noted superficial acute inflammation with formation of vesicles in the region of the crypts, sometimes spreading to surrounding structures.³⁹ This condition was present in 4 of 7 of his cases but has not been observed by other investigators.⁵¹

The pharynx was the site of acute fibrinous inflammation in 2 cases.¹⁴ In another instance the pharyngeal muscle was severely

47 Dragert, E. *Virchows Arch f path Anat* **292** 452, 1934

48 Mayer, A. *Deutsche med Wchnschr* **44** 857, 1918

49 Kaneko, R. *Ueber die pathologische Anatomie der Spirochaetosis ictero-haemorrhagica* Inada, Vienna, Rikola Verlag, 1922

50 Hecker and Otto. *Deutsche med Wchnschr* **18** 820, 1911. Beitzke⁴⁶

51 Dragert⁴⁷. Beitzke⁴⁰

damaged⁴⁷ The common occurrence of dysphagia suggests a greater frequency of lesions in this region

The bone marrow is unchanged⁴⁷

The skin either in the presence or in the absence of rash, shows cellular infiltration around the arterioles and capillaries of the cutis, following them into the papillae¹⁴ Lymphocytes and plasma cells but few polymorphonuclear leukocytes are present, in the epidermis wandering polymorphonuclear leukocytes or nests of them may occur beneath the horny layer These observations are not constant, however¹⁵

Spirochetes are distributed in the blood to every part of the body They have been observed in all the organs as well as the walls of arteries, muscles, lymph nodes, nervous system and internal ear Whereas in the guinea-pig they are extraordinarily abundant in the organs at the time of death, in man they are usually scarce Besides the liver and kidneys, the organs and tissues show the spirochetes in the following order of frequency adrenals, myocardium, intestinal wall, appendix, pancreas, prostate, lung, spleen, lymph nodes, skeletal muscle and wall of the bladder^{7,2} In the liver they may be seen in the interstitial tissue and the Kupffer cells, and in the later stages chiefly within the hepatic cells Their scarcity may be indicated by the result of one investigation in which long and exhaustive search yielded only 1 organism in sections of the liver taken from 5 patients and only 2 spirochetes in sections of muscle from 2 patients⁴⁰ In the kidney, on the other hand, they occur with considerable regularity in the lumen or within the epithelium of the convoluted tubules and persist there after their disappearance from all the other organs

A considerable variety of incidental conditions have been observed in cases of Weil's disease Many of them appear irrelevant Other complications, such as tonsillitis, bronchitis, pharyngitis, esophagitis or catarrhal enteritis, might conceivably have something to do with the original point of entry of the spirochetes It is more likely, however, that these inflammatory conditions are purely secondary lesions with more or less marked superimposed bacterial infection Demonstration of the primary point of penetration in the absence of trauma remains a challenge to the pathologist

CLINICAL PICTURE

The recognized concept of Weil's disease in foreign countries has been well described by Valassopoulos,^{13a} Dawson,^{13b} Inada,⁵⁸ Uhlenhuth,¹⁵ Schuffner²⁰ and many other investigators It has been customary to divide the disease into three stages, each with its characteristic features in regard to the behavior of the spirochetes in the blood, the anti-

52 Kaneko, R, and Okuda, K *J Exper Med* **26** 325, 1917

53 Inada, R *J Exper Med* **26** 355, 1917

bodies and the excretion of the organisms in the urine, as well as in respect to certain clinical signs and symptoms. A knowledge of these features is essential if one wishes to apply correctly the various diagnostic procedures. It is important to remember that in from 10 to 20 per cent of the cases the disease is so mild that the patient is hardly forced to bed¹⁰. Before the use of laboratory tests as a routine these mild cases were never recognized as instances of Weil's disease.

First or Febrile Stage—This stage is characterized by free circulation of spirochetes in the peripheral blood, lack of antibodies and absence of the organisms in the urine.

The disease is ushered in usually with a severe chill, followed by sustained high fever (102 to 104 F). Then occur severe headache (often with pain behind the eyes), muscular pains (usually in the legs and back) and marked prostration. In some of the severer cases the prostration may be out of proportion to the rest of the symptoms and dominate the clinical picture. Symptoms referable to the gastrointestinal tract may include anorexia, dysphagia, abdominal pain, nausea, vomiting and diarrhea. Occasionally there is cough or hiccup. Herpes labialis which becomes hemorrhagic is usually present. Varying cutaneous eruptions have been reported. Conjunctivitis is often striking and has been commented on by numerous writers as characteristic and important. Signs of meningeal irritation occur if the spirochetes enter the cerebro-spinal fluid. The white cell count is elevated, but only rarely is it more than 20,000 per cubic millimeter. This stage lasts from four to seven days.

Second or Toxic Stage—(This term has been substituted for the usual name "icteric stage," in order to correct the impression that jaundice is a necessary feature.) This stage is characterized by lack of spirochetes in the blood, development of antibodies and excretion of many organisms in the urine. Renal and hemorrhagic tendencies may be mild or absent in the cases in which jaundice is not shown.

Jaundice appears in 50 per cent of the cases and gradually deepens. The icteric index may reach 150 or more. A positive reaction to the direct van den Bergh test is expected, because hepatitis is largely responsible for the jaundice. An important triad of physical signs, particularly in cases of jaundice, is enlargement of the liver associated with absence of a palpable spleen and of generalized adenopathy. In rare instances swelling of the lymph nodes draining the site of the portal of entry of the organism may occur. Schuffner stressed the important prognostic dictum that among the groups of patients without jaundice there is practically no mortality. Most of the symptoms of the first stage decrease in intensity, and the fever falls by lysis. Hemorrhagic diatheses appear in the majority of cases (being more severe when jaundice is present) and may manifest themselves as

petechiae, epistaxis, subconjunctival hemorrhage, bleeding from the gums, hematuria, hemoptysis, melena or hematemesis. The patient becomes weaker and the heart action more feeble. Oliguria, with albumin, casts or red cells in the urine, is almost constantly present in the cases of more severe disease. The nonprotein nitrogen content of the blood is usually elevated and in fatal cases may reach 200 mg per hundred cubic centimeters. Anuria is present in many fatal cases. Death occurs in this stage and may be due to toxemia, cardiac insufficiency, renal failure with uremia, pulmonary edema or hemorrhage. The mortality rate ranges from about 5 per cent in Europe to as high as 50 per cent in Japan. The second stage lasts from seven to ten days.

Third or Convalescent Stage—This stage is characterized by the disappearance of spirochetes from the blood, their abundant excretion in the urine and complete development of antibodies in the blood.

The stage begins in the third week and is marked by gradual subsidence of all the earlier symptoms. Anemia and marked emaciation are commonly seen in the cases of severe infection and may last for months. A second fever, known as "after-fever," which lasts from four to twenty days, is seen in from about 25 to 40 per cent of the cases and starts usually during the third week of the disease. This fever was believed by Inada to be due to disintegrating toxins during the height of serologic immunity, he based this opinion on the fact that at the time of the fever there is no return of the principal symptoms and the blood is not infectious.

DIAGNOSIS BASED ON LABORATORY DATA

The blood or urine is examined according to the stage of the disease, it being remembered that the blood contains spirochetes during the first week only, or occasionally as late as the ninth day, and that the urine does not contain the organisms until about the tenth day, from which time they persist until as late as the thirtieth day. They have also been found in the cerebrospinal fluid during the first, and occasionally during the second, week.⁵⁴

Because serum treatment may be highly successful in the early stage and greatly diminished in effectiveness if delayed until the jaundice is fully developed,⁵⁴ early diagnosis is desirable. The patient may die during the time required for the disease to develop in an inoculated guinea-pig. Hence search for the organisms in the blood should be made at once. If this is unsuccessful, other tests must be utilized.

Examination of the Blood by Dark Field—This method is rarely successful with whole blood. Centrifugation of the blood three times

⁵⁴ Inada, R. Ido, Y., Hoki, R., Ito, H., and Wani, H. J. Exper. Med. 27: 283, 1918.

according to the method of Blanchard and Lefrou⁵⁵ increases the chances of success, yet often fails. It is difficult to throw down the spirochetes even at high speed. Good results may sometimes be obtained, however, simply by centrifugating at low speed sufficiently to precipitate the red corpuscles and examining a thick layer of the supernatant plasma. The spirochetes are said to be from ten to twenty times more readily detected by this means⁵⁶.

Examination of the Urine by Dark Field—After the tenth day this is often a successful method of diagnosis, but though the results are negative the evidence is not conclusive. The sediment from 50 cc of freshly voided urine should be used.

Inoculation of Guinea-Pigs—This means is the most satisfactory and is the usual method of establishing the diagnosis. What the mouse is to the pneumococcus, the guinea-pig is to the leptospira of Weil's disease. One injects intraperitoneally from 3 to 5 cc of blood (more than this may kill the animal prematurely) and 5 cc of cerebrospinal fluid or the sediment from 40 to 60 cc of freshly voided urine suspended in 5 cc of a physiologic solution of sodium chloride.

After a period of incubation of six days or more there is a sharp rise in temperature. As the peak is reached jaundice appears, and this is followed in about twenty-four hours by a fall in temperature, collapse and death. Necropsy reveals (1) jaundice of the skin and all the internal surfaces, (2) petechial hemorrhages in the skin, in the muscles of the abdomen and thigh, under the peritoneum and in the gastrointestinal mucosa, (3) multiple small, sharply defined hemorrhages in the lungs, especially through the lateral portion of the lower lobes, the spotted appearance of which suggests 'the wing of a mottled butterfly,'^{13b} (4) acute congestion of the kidneys with minute hemorrhages, and (5) large hemorrhages in the adrenals.

Spirochetes are readily demonstrated in dark field or india ink preparations of an emulsion of the liver and by impregnation of pieces of the kidney or liver by the Levaditi method. They may be cultivated or reinoculated into other guinea-pigs from the liver, kidney, urine or blood from the heart. Pigs may become ill of the disease and then recover. The pigs which do not die should therefore be killed and examined after from seven to ten days^{5b}. Still earlier results may be obtained by examining the animal's peritoneal exudate removed from day to day with a capillary pipet¹⁵.

55 Blanchard, M, and Lefrou, G. Bull. Soc. path. exot. **15** 699, 1922.

56 Ruys, A. Nederl. tijdschr. v. geneesk. **77** 3364, 1933, quoted by Schuffner¹⁰.

Culture of the Patient's Blood—In the early stage the blood may be cultured according to the method of Manteufel⁵⁷ To each of several tubes containing from 3 to 10 cc of sterile distilled water from 2 to 3 cc of the patient's blood is added, and the tubes are incubated three or four days at from 25 to 30 C Examination by dark field in positive cases will then show spirochetes in at least one of three or four tubes The organisms will live in this medium for three or four weeks

Preparation of Culture of the Spirochetes for Stock Use—This is best accomplished, according to Uhlenhuth and Fromme,¹⁵ by using a liquid medium containing sterilized tap water and rabbit serum Each of the narrow (1 cm) tubes is filled with 2.5 cc of water and 0.2 cc of fresh serum, the mixture is then inactivated for one hour at 60 C After inoculation with an emulsion of liver, blood from the heart or peritoneal exudate, paraffin is poured in, and the tubes are incubated at from 30 to 35 C or less The reaction must be neutral or slightly alkaline (the optimum p_H is 7.6, the maximum, 8.3) The use of hard water or saline solution is unfavorable Growth begins in from twenty-four to forty-eight hours and attains a maximum in from five to ten days¹⁵ Transplantation should be made of weakly growing strains every six days, with vigorous growths, every three weeks will suffice The medium used at present in the Netherlands was described by Davidson¹² A similar medium was successfully used by Korthof⁵⁸

Agglutination Procedures—Agglutination of known organisms with the patient's serum is not applicable as a diagnostic aid before from the sixth to the eighth day of the disease, at which time the antibodies first appear in the blood⁵⁹ The test may be performed with living or killed cultures Details of the technic used in the Netherlands were well described by Davidson¹² Dilutions of serum of from 1:10 to 1:100,000 are made, an equal volume of spirochetal emulsion is added, and examination is made by dark field after incubation for three hours at 32 C

The usual result with a serum giving a strongly positive reaction and living organisms is agglutination in dilutions as high as perhaps 1:300 and lysis (but no evident agglutination) in the higher dilutions (perhaps from 1:100 to 1:30,000) The use of a killed culture containing a 0.5 per cent solution of formaldehyde prevents lysis and permits agglutination in the highest dilution compatible with the strength of the serum (i.e., 1:30,000) There is also less danger in handling the culture

57 Manteufel, P. *Deutsche med. Wchnschr.* **47**:461, 1921

58 Korthof, G. *Zentralbl. f. Bakt. (Abt. 1)* **125**:429, 1932

59 Baermann, G., and Zuelzer, M. *Klin. Wchnschr.* **6**:979, 1927

According to Baermann,⁵ the titer on the first day on which a positive result appears is already 1:100, it rises quickly in the next four to six days to 1:1,200 and in the course of the next ten to fourteen days to from 1:10,000 to 1:50,000 and higher. The height of the titer is independent of the severity of the illness, and the serums will almost always agglutinate at the same time other strains of *Leptospira*, often to an appreciably greater extent than the homologous one.

During the first fifty days after onset of the disease the agglutinating power is very high, and, in general, titers between 1:10,000 and 1:50,000 (with an average of 1:34,000) are observed. The titer falls rapidly to about 1:700 during the next fifty days and then declines gradually, averaging 1:300 between the three hundredth and the nine hundredth day. The agglutinating power of the serum is entirely negative in about a third of the cases from the two hundredth day on.³⁷

The agglutination test is useful in diagnosing cases in which previous infection is suspected as well as those of the acute state of the disease, and it is important to realize that a positive reading in dilutions of the lower hundredths during an acute illness stimulating Weil's disease may really be due to an old attack and may thus lead to false diagnosis. Gaetgens reported an apparently false positive reading in a case of infection with the paratyphoid B bacillus which is perhaps to be explained on this basis.⁶⁰ A carefully determined history and failure of the titer to rise should reveal the true situation.

Agglutination with the dog strain (*L. canicola*) may advantageously be attempted, as well as examination of the urine of any dog suspected of being a vector. For specific agglutination of this kind Castellani's absorption test with a culture treated with formaldehyde is used.⁶¹

The Pfeiffer Phenomenon—This test may be utilized for aid in diagnosis and for approximate titration of the patient's serum. One cubic centimeter of an emulsion of spirochetes mixed with an equal amount of serum is injected intraperitoneally into a guinea-pig weighing 200 Gm (or less), and the peritoneal fluid is examined by dark field after one and one-half hours. The highest dilution of the serum which will still produce complete lysis of the organisms is subsequently determined. Tests may be carried out similarly to find the smallest amount of serum which will protect a guinea-pig from infection by 1 cc of culture given intraperitoneally. (Inada found that 0.01 cc of the horse serum which he used would accomplish this result and that it was as strong as convalescent serum.³⁶) Unprotected guinea-pigs used as controls die within

⁶⁰ Gaetgens, W. *Klin Wchnschr* **12** 697, 1933.

⁶¹ Castellani, A., and Chalmers, A. *Manual of Tropical Medicine*, ed 3, New York, William Wood & Company, 1919, p 1397. Schuffner¹⁰

the usual time (from five to twelve days) The adequately protected pigs survive this period, but some may die later (from the fourteenth to the nineteenth day) ⁶²

Complement-Fixation Test—This procedure, with antigen made from a culture of the spirochetes, has been used with marked success by Gaeltgens ⁶⁰ With 70 unknown serums the results of the agglutination and complement-fixation tests coincided perfectly, 29 giving positive reactions Higher titers were obtained by agglutination tests, but complement-fixation gave more clearcut negative readings in some cases in which agglutination was questionable in the lower dilutions The test may be of special value in early diagnosis The average titer in 5 cases on the seventh and eighth days of the disease was strongly positive (1:300) The test also constitutes a valuable confirmatory check on the results of agglutination

Precipitation Test—Hindle and White ⁶³ isolated a specific soluble substance from the spirochetes which in solution gave precipitation in dilutions of the antiserum of the homologous spirochete Details of this method have not to our knowledge been published ⁶⁴

Adhesion Test—Recently this method of diagnosis was proposed by Brown, ⁶⁵ who stated that the reaction to this test was easier to read than the agglutination reaction and that the test could be performed more rapidly and was equally sensitive and specific The adhesion test is based on the principle that particles, such as bacteria or blood platelets, become adherent to the spirochetes in the presence of immune serum The details of the technic are well described in the paper just mentioned The indications for its use are the same as those for the agglutination test

COMMENT ON THE PRESENT CASE

The clinical history, physical findings and laboratory data were typical of Weil's disease The occupation (fish cutting) should in itself have aroused suspicion In consideration of the differential diagnosis Weil's disease was mentioned as a rare possibility Unfortunately at that time the value of laboratory procedure was not appreciated, and a definite diagnosis was not made until after the necropsy It is even conceivable that had an immediate diagnosis been made and serum been available the patient might have been saved

The record of the necropsy in our case is the first complete pathologic study of an uncomplicated case of Weil's disease to be reported in this

62 Ido, Y, Hoki, R, Ito, H, and Wani, H J Exper Med 24 471, 1916

63 Hindle, E, and White, P B, in discussion on Schuffner ¹⁰

64 Lusena, M, and Crainfanti, E Soc internaz di microbiol, Boll d sez ital 6 77, 1934

65 Brown, H C Brit M J 1 411, 1935

country. The American literature contains the results of three earlier postmortem examinations. McDowell^{5a} limited his description to a few lines, while both of Ball's⁹ cases were complicated by other diseases.

This pathologic study illustrates that remarkably normal histologic conditions in the liver are possible in a patient dying as early as the end of the first week. Absence of biliary stasis and lack of evidence of increased destruction of red cells in the spleen or bone marrow fail to throw further light on the mechanism of the production of jaundice.

The high content of nonprotein nitrogen (200 mg) in the blood of this patient was unusual with so little apparent damage of the renal parenchyma, but the debris in the tubules was evidence of a not inconsiderable preexisting necrosis of epithelium. Hepatitis may have been responsible for the low content of cholesterol found in the blood (90 mg per hundred cubic centimeters). The high value for phosphorus (13.6 mg) was checked by duplicate determination. We are unable to explain it.

Necrosis of certain isolated fibers in the heart is believed to be a specific result of the spirochetosis. Acute esophagitis has not to our knowledge been previously described in this disease.

COMMENT ON THE AMERICAN CASES

The available data on the reported American cases have been compiled in two tables. It can be readily seen that each case presents a clinical picture which, in spite of minor variations, resembles the classic European and Japanese types of the disease. With 1 exception (the accidental infection of a woman technician), all the cases occurred in men of middle age. In no instance was there an observed secondary case. The onset of the disease occurred during any season of the year. Occupations (not listed) were of interest in that the group of patients included a sewer worker, a member of a swimming team, a cook, a laborer and a fish cutter. Contact with rat-infested buildings was mentioned several times. Also in 1 instance the disease developed after the patient had been soaked in rain-water.

Jaundice and prostration occurred in all cases. This association is interesting in view of the fact that over one half of the European cases occurred without jaundice, it probably means that the nonicteric cases are not recognized in this country. Chills, fever, headache, muscular pain, vomiting and hemorrhagic tendencies were almost constantly present. Enlargement of the liver and absence of palpable spleen and lymph nodes were rather constant physical signs. Dysphagia, cough, hiccup, herpes, pharyngitis and conjunctivitis were minor signs and symptoms. In 83 per cent of the cases there were characteristic findings in the urine. When recorded, the nonprotein nitrogen content of the blood

was definitely elevated in all but 1 case, in which, curiously enough, no changes in the urine were shown

Weil's disease being spirochetal, the Wassermann reaction might perhaps be expected to be influenced, but it was consistently negative

TABLE 1—General Information and Laboratory Data in Cases of Weil's Disease Reported in the United States

Case	Author Reporting Case	Age, Years	Sex	Month of Occurrence of Disease	Laboratory Data					
					Non protein Nitrogen, Mg	Albu min	Urine		Wasser mann Reaction	White Cell Count
							Red Cells	Casts		
1	Wadsworth	?	F	Feb	—	0	0	0	Negative	+
2	McDowell	43	M	July	—	+	+	+	Negative	—
3	Sailer	51	M	Aug	—	+	0	0	Negative	—
4	Sailer	44	M	Oet	—	+	+	+	—	Normal
5	Hayman and Lynch	42	M	Oet	73	+	0	+	Negative	10,300
6	Towler and Walker	31	M	Dec	83	+	+	+	Negative	19,000
7	Mulholland and Bray	20	M	Feb	—	+	0	+	Negative	14,200
8	Cushing	35	M	Sept	46	+	0	0	Negative	10,400
9	Cushing	37	M	Oet	30	0	0	0	Negative	11,600
10	Ball	51	M	Jan	162	+	+	+	Negative	14,200
11	Ball	42	M	Nov	—	+	0	+	Negative	9,000
12	Jeghers, Houghton and Foley	38	M	May	200	+	0	+	Negative	28,750

* In this table — indicates that data were not available, +, present or increased

TABLE 2—Clinical Signs and Symptoms in Cases of Weil's Disease Reported in the United States*

Signs and Symptoms	Case												Percentage of Positive Findings
	1	2	3	4	5	6	7	8	9	10	11	12	
Initial chill	?	?	+	+	+	+	0	+	+	?	+	+	65
Fever	+	+	+	+	+	0	+	+	+	+	+	+	91
Headache	0	0	+	+	+	+	+	+	+	?	?	+	65
Muscular pains	?	+	+	+	+	+	+	+	+	?	?	+	74
Prostration	+	+	+	+	+	+	+	+	+	+	+	+	100
Conjunctivitis	0	0	0	+	0	+	0	0	0	0	0	+	25
Injection of pharynx	0	0	0	0	+	+	+	0	0	0	0	0	25
Herpes	0	0	0	0	+	0	0	+	0	0	0	+	25
Vomiting	+	+	0	+	+	+	0	+	0	+	0	+	65
Hiccup	0	+	0	0	+	0	0	0	0	0	0	+	25
Icterus	+	+	+	+	+	+	+	+	+	+	+	+	100
Cough	0	0	0	0	+	+	0	0	0	+	0	0	25
Hemorrhagic tendencies	0	+	0	+	+	+	+	+	0	0	0	+	58
Enlarged liver	0	0	+	+	+	+	+	+	+	0	0	+	65
Palpable spleen	0	0	0	0	0	0	0	0	+	0	0	0	8
Adenopathy	0	0	0	0	0	0	+	0	0	0	0	0	8
Outcome	R	D	R	R	D	R	R	R	R	D	D	D	% dead 41

* In the table, 0 indicates absence of the symptom, +, its presence, R, recovery of the patient, D, death, and ?, no mention

It is of interest to note in this connection that Edelman⁶⁶ recorded a positive Wassermann reaction among his cases of rat-bite fever a spirochetal diseases due to *Spirochaeta morsus-muris*

66 Edelman, S D, and Haber, G B J Pediat 5 520, 1934

The diagnosis was verified in all instances by examination of the blood by dark field, by inoculation of guinea-pigs or at necropsy. Forty-one per cent of the cases terminated fatally. This seems a high rate of mortality, but it is best explained on the supposition that the milder cases without jaundice (in which the mortality rate is low) were not diagnosed.

DIFFERENTIAL DIAGNOSIS

Two common types of jaundice exist in this country which are confused with each other and with Weil's disease. 1 Infectious jaundice, as Blumer,⁶⁷ Wadsworth¹ and other investigators have shown, occurs as a benign and highly contagious disease in this country. The etiology is unknown, and the study of hundreds of cases has not once shown it to be due to a spirochete. It is characterized by appearing in epidemic form, mostly in the fall and winter, and by attacking primarily children and adolescent boys and girls. It is rarely fatal and lacks the renal and hemorrhagic manifestations of Weil's disease. 2 Catarrhal jaundice clinically resembles the afebrile type of infectious jaundice and is looked on by some investigators as the sporadic form of this disease. However, Held and his associates⁶⁸ pointed out that it is essentially a degenerative involvement of the polygonal hepatic cells, which are rendered susceptible by loss of glycogen to the action of an as yet unknown toxic substance. Softer⁶⁹ showed that one attack may leave permanent damage to the liver. It is rarely fatal, it is noncontagious and attacks primarily persons under the age of 40.

The possibility of acute yellow atrophy may be confusing but should be ruled out on the basis of the clinical course, shrinkage of the liver and the finding of leucine or tyrosine crystals in the urine.

When jaundice is absent (or before it appears) Weil's disease may simulate trichiniasis (muscular pain and ocular signs), nephritis (renal injury), dyscrasias of the blood (hemorrhagic tendencies), severe generalized infection (chill, fever, malaise and prostration), meningitis (headache and stiff neck) and gastro-enteritis or enteric fever (nausea, vomiting, abdominal pain or diarrhea).

Relation to Yellow Fever—Weil's disease has been confused with yellow fever because of the isolation of *Leptospira* by Noguchi from the blood of patients with supposed yellow fever and because of the extraordinary similarity of the clinical syndromes. It has been conclusively shown, however, that Noguchi's organism and the *leptospira* of Weil's

⁶⁷ Blumer, G. J. A. M. A. **81** 353, 1923.

⁶⁸ Held, I. W., Goldbloom, A. A., and Kramer, M. L. Internat Clin **4** 197, 1931.

⁶⁹ Softer, L. J., and Paulson, M. Arch Int Med **53** 809, 1934.

disease are serologically identical⁷⁰ The blood of patients with yellow fever is pathogenic primarily for *Macacus rhesus* rather than for the guinea-pig Yellow fever convalescent serum protects the monkey from the virus of yellow fever but has no effect on *L. icterohaemorrhagiae*, whereas convalescent or immune horse serum of Weil's disease protects against the spirochetes (agglutinates and dissolves them) and fails to protect against the virus of yellow fever The obvious and generally recognized explanation, therefore, is that the spirochetes in the material received by Noguchi were obtained from patients with Weil's disease contracted by accident during the outbreak of yellow fever Two such instances have since occurred in the epidemic in 1928 in Rio de Janeiro⁷¹ No spirochete has ever been recovered from patients with West African yellow fever, and the disease has finally taken its place among those attributed to a filtrable virus

Schuffner convincingly showed that the decision of "Weil or no Weil" rests with the bacteriologist and the pathologist The bizarre symptomatology and marked variation in severity of Weil's disease have caused considerable confusion and have made its recognition on purely clinical grounds often impossible Hence, in all suspected cases the diagnosis should be established or rejected by means of the laboratory procedures previously described

TREATMENT

As long as the disease is rare and sporadic in this country treatment will be symptomatic However, it should be recognized that a highly potent serum has been developed in Europe and Japan With serum treatment, as noted by Inada there are reduction in the mortality rate distinct amelioration of the symptoms and shortening of the course of the disease The polyvalent serum of Baermann and Smith produced a surprising effect even in the severest, most critical cases or even when given relatively late The patients often felt better in a few hours³¹ Instances of surprisingly successful results from serum treatment in Weil's disease have repeatedly been observed in the Netherlands⁷²

The serum should be given preferably during the first few days intravenously and in large doses Inada gave 60 cc in twenty-four hours, 40 cc the first day and 20 cc the next or 20 cc on each of three consecutive days⁵⁴ Baermann gave from 60 to 90 cc the first day and then from 30 to 40 cc every other day until a total of 200 cc was

⁷⁰ Theiler, M., and Sellards, A. W. *Am J Trop Med* **6** 388, 1926, **7** 369, 1927

⁷¹ Muller, H. R., and Tilden, E. *J A M A* **94** 856, 1930

⁷² Schuffner, W., and Mochtar, A. *Zentralbl f Bakt (Abt 1)* **101** 405, 1927

used in very severe cases. No unfavorable reaction of consequence was even noted.

Serum is positively indicated, if available, for patients with severe symptoms whose disease has been diagnosed and for laboratory workers with a clear history of accidental exposure several days or more before the onset of symptoms.⁷⁰ "In any event," wrote Uhlenhuth, "according to all past experience, experimental and clinical, the early use of large doses of specific serum must be urgently recommended for the treatment of Weil's disease."⁷¹

Because of the heterogeneity of the various strains of *Leptospira* (as indicated by minor differences in titer required for agglutination), the serum should be widely polyvalent. Noguchi used nine strains from North and South America, Japan, England and France, yet his serum failed to affect certain other strains. Baermann used one hundred Sumatran strains and fifteen others, including strains from the Netherlands, Germany and France. Organisms of all degrees of virulence were represented.⁷²

Standardization of the serum must for the present be based on the agglutination or lysis titer of existing fresh virulent strains. Baermann's noteworthy results were obtained with serums the titer of which ranged from 1:1,000 to 1:10,000.

Rapid production of serum for use in emergency (as in an epidemic) was described by Uhlenhuth.¹⁵ He found that adult rabbits given a single large dose of organisms survived the period of jaundice and produced antibodies of considerable value much earlier than they were produced in the horse or sheep. Properly preserved serum should be efficacious after several years of storage. That of Griffith, which contained 0.4 per cent phenol and was kept in a cool dark cupboard, maintained its original potency for seven years.⁷³

Equally good results have followed the use of convalescent serum obtained from patients recovered from the disease in whom high agglutinating and lytic titers are known to have developed. It would be an excellent plan if in the future convalescent serum were collected from each person recovering from Weil's disease in this country.

Arsphenamine and other arsenical preparations have been shown by numerous clinicians to be ineffective as a spirocheticidal agent in Weil's

73 As far as we have been able to determine, serum is not at present available in this country. Manufacture of Noguchi's serum was discontinued after it was found nonspecific for yellow fever. According to Fairley,¹¹ serum is obtainable from Burroughs, Wellcome & Co., London, and we suggest that it might be procurable through their New York agency.

74 Griffith, A. J. Hyg. 18: 59, 1919.

disease. They may further damage the liver. A soluble preparation of bismuth (bismuto-chinioform) has been found remarkably effective in guinea-pigs⁷⁵. Although no reports of its use in man are available in the literature, it seems worthy of trial in severe cases if serum is not available. The symptomatic treatment is essentially similar to that for hepatitis, nephritis or severe generalized infection.

GENERAL COMMENT

In a recent editorial in *The Journal of the American Medical Association* it was stated that "Weil's disease seems to be one of those conditions fated to play an ever larger part in medicine and public health"⁷⁶. Evidence has been brought forward in this paper which we believe amply substantiates this prophecy.

Certainly, the same potential factors for the spread of the disease exist in this country as in other countries, and it has been shown that (1) the Weil strain of *Leptospira* is the same the world over, (2) at least 10 per cent of wild rats in the United States harbor and excrete virulent organisms, (3) occupational exposure exists (most of the 12 reported cases occurred in persons working at certain trades), (4) spirochetes have been found to thrive in water in many places in this country, and (5) sporadic cases of Weil's disease (clinically and pathologically identical with the classic European variety) continue to be reported at intervals.

That lack of recognition of the disease accounts for the report of so few cases in the United States has been commented on. A condition comparable to that obtaining in this country existed in Scotland, France, the Netherlands and other countries about ten years ago, when Weil's disease was rarely reported to the health authorities. During recent years (owing almost entirely to a better understanding of this disease entity), it has become commonly recognized. Physicians in the aforementioned countries consider Weil's disease frequently in their differential diagnoses. It has become as common there to send blood to the health laboratories for agglutination with *Leptospira* as it is in this country to send blood to a state laboratory for a Widal test. A typical example is the Pasteur Institute of Paris, where during 1933, 1,232 specimens of blood from persons suspected of having Weil's disease were examined, and agglutination with *Leptospira*⁷⁷ was obtained in 23.1 per cent.

⁷⁵ Uhlenhuth, P., and Seiffert, A. *Zentralbl. f. Bakt. (Abt. 1)* **114** 241, 1929.

⁷⁶ Editorial, *J. A. M. A.* **103** 493, 1934.

⁷⁷ Erber, B. *J. Bull. Office internat. d'hyg. pub.* **24** 1749, 1934, abstr., *Brit. M. J.* **2** 1155, 1934.

The true incidence and future course of Weil's disease in America will be better determined if the following conditions are fulfilled

- 1 The disease must be suspected more often and the variation in symptomatology appreciated

- 2 All suspected cases should have laboratory confirmation

- 3 Laboratories (both public health and hospital) should be prepared to make the necessary diagnostic tests

- 4 Serum and prophylactic measures should be utilized when indicated

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc—At the University of Michigan Malcolm H Soule, professor of bacteriology, has been made director of the hygienic laboratory and chairman of the department of bacteriology in succession to F G Novy, who has retired

Ernest E Tyzzer, professor of bacteriology in the Harvard University Medical School, has received the honorary degree of doctor of science from Brown University

C R Bardeen, professor of anatomy and dean in the medical school of the University of Wisconsin, has died at the age of 64

Walter J Nungester, assistant professor of bacteriology in the medical school of Northwestern University, has been appointed associate professor of bacteriology in the University of Michigan

Albert C Broders, of the Mayo Clinic, has been appointed professor of surgical pathology and director of cancer research at the Medical College of Virginia, Richmond

Broman C Crowell, director of clinical research in the American College of Surgeons, Chicago, has been elected president of the Gorgas Memorial Institute

A R Dochez has been elected a member of the board of scientific directors of the Rockefeller Institute for Medical Research

John Weinzerl, professor of bacteriology in the University of Washington since 1912, has died at the age of 65

The Poor Richard Club of Philadelphia has awarded its medal of achievement to John A Kolmer in recognition of his work on prevention of epidemic poliomyelitis

N Paul Hudson, professor of pathology in the University of Chicago, has accepted an appointment as professor and chairman of the department of bacteriology in Ohio State University, in place of Charles B Morrey, who has resigned

Floyd S Markham, J M Birkeland and O C Woolpert have been appointed assistant professors of bacteriology in Ohio State University

A bronze plaque of Alfred S Warthin, who died in 1931, has been presented to the University of Michigan by persons who worked with him in the department of pathology under his directorship Dr Warthin was connected with the university from his graduation there in 1891, since 1903 and until his death he was professor of pathology and director of the pathologic laboratory

Ralph S Muckenfuss, assistant professor in Washington University, St Louis, has been appointed temporary assistant director of the bureau of laboratories of the department of health of New York City

Society News—At the annual meeting of the American Society of Clinical Pathologists Roy R Kracke was chosen president-elect, R A Kilduffe, vice-president, and A S Giordano, secretary-treasurer

The forty-sixth annual meeting of the Association of American Medical Colleges will be held at Toronto, Canada, on Oct 28, 29 and 30, 1935

Corrected Notice—The Second International Congress for Microbiology will be held in London July 25 to Aug 1, 1936

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

PURE CULTURES OF FIBROBLASTS FROM SINGLE MONONUCLEAR CELLS J K MOEN, J Exper Med **61** 247, 1935

Most guinea-pig mononuclear exudative cells in tissue culture become typical migrating macrophages, but a small proportion take on fibroblastic characteristics and produce pure colonies of fibroblasts. These fibroblasts maintain their morphologic characteristics through repeated subcultures. It is suggested that the development of individual mononuclear cells in tissue culture subsequent to isolation is conditioned at the time of explantation. Apposition to other cells is not necessary for the initiation of mitotic cellular division. There is a definite optimal relationship between the bulk of the medium, the number of explanted cells and the extent of proliferation. The presence of other cells in the vicinity enhances cellular division. Mitosis in the isolated explanted cell is preceded by a latent period. The rate of division varies in different colonies of fibroblasts. Added erythrocytes in the mononuclear suspension definitely inhibit proliferation of fibroblasts in tissue culture. The inhibiting factor in disintegrating erythrocytes is apparently in the stroma.

FROM THE AUTHOR'S SUMMARY

BLOOD PLASMA PROTEIN REGENERATION CONTROLLED BY DIET W T POMMEL-RENKE ET AL., J Exper Med **61** 261 and 283, 1935

1 *Standardization of Food Proteins, Fasting and Iron Feeding*—When blood plasma proteins in dogs have been depleted by bleeding, with return of washed red cells (plasmapheresis), it is possible to bring the animals to a steady state of low plasma protein and uniform production of plasma proteins on a basal diet. Such dogs are excellent test subjects by which to measure the potency of various dietetic factors for the regeneration of plasma proteins. To regenerate plasma proteins in any significant amount the depleted dog requires food proteins. Some proteins are very potent for the production of new plasma proteins, and others are utilized poorly. Beef serum is potent, the proteins in 2.6 Gm of serum will produce 1 Gm of new plasma proteins in the depleted dog—a potency ratio of 2.6. Kidney protein stands at the bottom of the list, the dog needs 21 Gm of kidney protein to regenerate 1 Gm of plasma protein—a potency ratio of 21. Some grain proteins approximate beef serum in potency and may show potency ratios of from 2.7 to 4.6. Some of these grain proteins appear to favor the production of globulin more than that of albumin in the plasma. Skeletal muscle, gizzard (smooth muscle), lactalbumin and egg-white fall into a favorable group with a potency ratio of from 5.3 to 6. Whole liver, liver fractions, casein and beef heart are a little less potent, presenting potency ratios of from 6.5 to 8. Many of these food substances favor the production of albumin more than that of globulin. Pancreas and salmon muscle show less favorable potency ratios of 19 and 15, respectively. Fasting periods indicate that the depleted dogs can produce little if any new plasma protein. Iron feeding in some unexplained manner influences body metabolism so that an excess of plasma protein is produced. These observations have a bearing on clinical conditions associated with hypoproteinemia and give suggestions for dietary aid or control in some of these abnormal states. The make-up of the diet is obviously of great interest, and it is possible that combinations of proteins may be more potent than a single protein or that food potency ratios may differ in health and disease.

2 Dog Plasma Protein, Horse Plasma and Dog Hemoglobin—Foreign plasma protein (horse) introduced parenterally into the dog deprived of dietary protein is not utilized in the body economy. Its fate appears to be disintegration and elimination as excess urinary nitrogen. This is totally different from the fate of dog plasma protein under similar conditions. Dog hemoglobin given parenterally to the dog deprived of protein is not utilized as is dog plasma protein to keep the animal in nitrogen equilibrium, the globin is largely broken down and discarded as excess urinary nitrogen. A small part of the injected hemoglobin is probably utilized to maintain the red cell concentration in the blood at high levels. Dog plasma given parenterally in a dog deprived of dietary protein will maintain the dog in nitrogen equilibrium, and there is no surplus nitrogen elimination in the after-periods. It is apparent that the introduced plasma protein is utilized efficiently in body metabolism to replace or repair tissue protein. It is suggested that although this is an emergency reaction the same reaction may go on in normal internal metabolism. The observation that foreign plasma and dog hemoglobin cannot be utilized when given parenterally strengthens the argument for a normal contribution from plasma proteins to body proteins.

FROM THE AUTHORS' SUMMARIES

RENAL DAMAGE FROM A DIET CONTAINING EXCESS INORGANIC PHOSPHATE
E. M. MCKAY and J. OLIVER, *J. Exper. Med.* **61** 319, 1935

The addition of an excess of inorganic phosphate in the form of orthophosphoric acid, acid, basic or neutral sodium or potassium phosphate to the diet of albino rats results in the development of an interesting and permanent renal lesion. The lesion is characterized by necrosis of the cells of the convoluted tubules commencing at the terminal end, followed by atypical regeneration of epithelium and calcification of the necrotic debris that fills the tubules. The entire outer stripe of the outer zone of the medulla is transformed into a zone of distorted structures, and there is an increase in the interstitial connective tissue. The adjoining cortex is also involved with cystic dilatation of tubules and collapse. Such areas may reach the free surface of the organ and produce retracted scars. In the gross the kidneys are enlarged and firm on section, with a pebbled surface produced by numerous scars. The maximum changes in the kidney structure are reached after about fifteen days, but necrosis of the cells of the convoluted tubules is evident after a single day of phosphate feeding. The renal structure is not restored to its normal form when the excess of phosphate is removed from the diet.

FROM THE AUTHORS' SUMMARY

MOTTLED ENAMEL IN CATTLE H. T. DEAN, *Pub. Health Rep.* **50** 206, 1935

An additional area, west Texas, showing mottled enamel in cattle is reported. The economic consequence of widespread fluorosis in stock may be a problem of some significance in animal husbandry.

FROM THE AUTHOR'S SUMMARY

THE ACTION OF ACETONE AND OF THE KETONE BODIES PRESENT IN DIABETIC BLOOD UPON THE HEART M. M. BAGOURY, *Brit. J. Exper. Path.* **16** 25, 1935

Different concentrations of the ketone bodies present in diabetes were compared as to their action on the heart muscle and the minimal effective doses determined. Acetone and aceto-acetic acid produce a weakening of the heart, as evidenced by the cardiac dilatation and the rise of the venous and pulmonary pressures. The toxicity of aceto-acetic acid is about from fifteen to twenty times greater than that of acetone. The minimal effective concentration of aceto-acetic acid is from 2 to 3 mg. and that of acetone from 25 to 40 mg. per hundred cubic centimeters of blood. The weakening effect of acetone remains unchanged so long as the acetone remains in the circulation. On replacing the blood by acetone-free blood the heart recovers completely. The cardiac dilatation produced by

small doses of aceto-acetic acid tends to disappear spontaneously. Beta-oxybutyric acid has no specific action on the heart up to concentrations of 1 per cent. Its effects are entirely accounted for by the changes in the acid-base equilibrium which the acid produces in the blood. The three ketone bodies produce an increase in the coronary blood flow. This effect is, however, negligible unless the concentration of these substances in the blood is very high. Administration of dextrose and of insulin does not modify the action of these three substances on the heart muscle.

FROM THE AUTHOR'S SUMMARY

Pathologic Anatomy

HISTOLOGICAL EFFECTS OF POTASSIUM IODIDE AND THYROID SUBSTANCE ON THE THYROID GLAND OF THE GUINEA PIG IN SCURVY. W. F. ABERCROMBIE, *Am J Path* **11** 469, 1935

In scurvy the thyroid gland presents irregular follicles with higher epithelium, a reduced amount of nonuniformly stained and extensively vacuolated colloid and an increase in the interfollicular cells. These changes are more marked in chronic scurvy of long duration than in acute scurvy. Potassium iodide causes a decrease in the number of vacuoles and an increase in the amount of colloid, accompanied by a flattening of the epithelium and a decrease of the interfollicular cells. Thyroid substance produces similar results except that the epithelium is not flattened but is returned to the normal medium height. Potassium iodide and thyroid substance, in the doses administered, do not tend to prolong the life of the scorbutic animal. Thus, it appears that vitamin C is not concerned with iodine metabolism.

FROM THE AUTHOR'S SUMMARY

GLOMERULAR CHANGES IN ARTERIOSCLEROTIC CONTRACTION OF THE KIDNEY. P. KIMMELSTIEL, *Am J Path* **11** 483, 1935

In arteriosclerotic kidneys the following degenerative changes can be recognized in the glomeruli:

A. Primary broadening and hyalinization of the intercapillary axial connective tissue. This very frequent change is interpreted as a phenomenon of the aging of the glomerulus and may lead to damage of the glomerular capillaries.

B. Thickening of the basement membrane, which is always secondary. It may be due to two different causes:

1. Ischemic atrophy of the glomerulus, which may result from (a) direct encroachment of hyalinization of the vas afferens on the glomerulus leading to collapse and degeneration of all the glomerular elements, and (b) narrowing of the larger vessels producing slow circulatory atrophy of the tubules and glomeruli. This change, the most common in all forms of arteriosclerotic kidney, is characterized by thickening of the capsule and basement membrane, frequently extending from the former to the latter. This thickening of the capsule is closely associated with atrophy of the tubular epithelium.

2. Ascending atrophy. This is caused by obstruction of the corresponding tubules and is characterized by thickening of the capillary basement membrane without thickening of the capsule and is associated with dilatation of the capsular space. This form usually is not observed in pyelogenic ascending contraction. The latter is interpreted mainly as an ischemic process, thereby explaining the fact that in this condition one so frequently encounters a high degree of capsular thickening (vide 1b).

Tubular atrophy in arteriosclerotic kidneys is chiefly circulatory and essentially depends on changes in medium-sized and larger vessels.

FROM THE AUTHOR'S CONCLUSIONS

BLOOD-FILLED CYSTS ON THE CARDIAC VALVES IN INFANCY S D MILLS, *J Pediat*
6 51, 1935

Small blood-filled cysts occur not infrequently on the leaflets of the cardiac valves in the neonatal period. The best explanation for their origin is that they are cystic dilatations at the ends of endothelium-lined canals in the substance of the valves.

FROM THE AUTHOR'S SUMMARY

THE HISTOLOGY OF THE TUBERCULOUS CAVITY WATT S R GLOYNE, *Tubercle*
16 161, 1935

In the cavity of the acute bronchopneumonic tubercle the connective tissue is minimal, and the parenchyma of the lung still forms the greater part of the wall. In the old cavity with a thick wall the latter consists entirely of dense connective tissue which has obliterated all other structures with the exception of bronchi which contain portions of cartilage enabling them to resist compression. Between these two extremes are those which may be designated the cavities of the intermediate stage. No single description will fit the walls of all, their structure is varied. There is always more connective tissue in the wall than examination with the naked eye suggests. Setting aside the somewhat rare small cavity of the acute bronchopneumonic tubercle, one may safely infer that by the time a cavity is recognizable in the roentgenogram connective tissue is already formed in its walls.

H J CORPER

THE LESIONS OF THE CHRONIC FORM OF PERIARTERITIS NODOSA MACAIGNE and
 P NICAUD, *Ann d'anat path* **11** 235, 1934

In an adult with recurrent attacks of asthenia, polyneuritis, polymyositis and multiple nodules of the skin of about ten years' duration biopsies of the nodules revealed arterial and arteriolar changes considered characteristic of periarteritis nodosa: endothelial proliferation with thickening of the intima and narrowing of the lumen, doubling of the elastica interna, separation of the muscle fibers of the media and infiltration of the adventitia by granulation tissue and fibrosis. Foci of necrosis and occasionally suppurative changes were present. Inoculation of material from the lesions into two guinea-pigs and a monkey yielded no results.

PERRY J MELNICK

PRIMARY POLYMYOSITIS G MARINESCO, S DRAGANESCO and E FAÇON, *Ann d'anat path* **11** 537, 1934

The authors report a case of primary hemorrhagic nonsuppurative polymyositis (Frenkel-Wetzoldt). Microscopically the muscles showed extensive degenerative changes but also marked inflammatory changes and hemorrhages. Various fixatives and stains were used so that artefacts were not a confusing element. Different degrees of degenerative changes progressing to coagulation and necrosis were seen. The inflammatory process had the character of an interstitial subacute infiltration of round cells, capillaries and connective tissue. The authors consider the condition to be inflammatory, and differentiate it from muscular dystrophies, myasthenia gravis, Landry's syndrome, rheumatism, trichinosis, neuromyositis and dermatomyositis.

PERRY J MELNICK

HISTOLOGIC STRUCTURE OF THE REMAINING PORTION OF THE THYROID AFTER CURE
 OF EXOPHTHALMIC GOITER BY SUBTOTAL THYROIDECTOMY G ROUSSY,
 R HUGUENIN and H WELTI, *Ann d'anat path* **11** 555, 1934

In two cases the authors made biopsies of the portion of thyroid gland remaining after subtotal thyroidectomy for thyrotoxicosis, in one case two years, in another case five years, after the operation. The specimens were obtained during cosmetic

repair of the scars. In both cases the histologic picture was identical with that of the goiters previously removed. In both, although the organ was clinically cured, with normal physiology reestablished, the thyroid tissue was found to be as hyperplastic as the original goiter.

PERRY J MELNICK

STUDIES OF THE INTERMEDIATE LOBE OF THE HYPOPHYSIS G ROUSSY and M MOSINGER, *Ann d'anat path* **11** 655, 1934

The authors made a histologic study of the hypophyseal fissure, the diverticula of the fissure, the sero-albuminous glands of the intermediate lobe and the cellular infiltrations of the posterior lobe. They studied 112 normal hypophyses from adults of various periods of life ranging to advanced age, 6 from infants and children, and 1 from an 8 month fetus. In the fetus and the infant the hypophyseal fissure is intact. In both the anterior and posterior walls the lining epithelium is the same. Therefore the anterior wall properly belongs to the intermediate lobe. Besides flat, cuboidal and cylindric cells a fourth type of cell is found in this epithelial layer, namely, a basophilic branched cell apparently capable of migration. From the fissure of the hypophysis diverticula branch off in all directions, including diverticula into the posterior lobe. These are different from the sero-albuminous glands of Erdheim in the intermediate lobe, which are lined by a different type of secreting epithelium. Cellular infiltrations into the posterior lobe consist, in infants, of basophilic cells from the intermediate lobe, but in adults they may also consist of eosinophilic cells from the anterior lobe since the fissure is obscured and there is a more direct continuity with the anterior lobe. These anatomic structures point the way to interesting conclusions regarding the various physiologic and pathologic states of the hypophysis.

PERRY J MELNICK

CHANGES IN THE ENDOCRINE GLANDS IN DIABETES MELLITUS M LABBE and M PETRESCO, *Ann d'anat path* **11** 761, 1934

The hypophysis, thyroid and adrenals were studied histologically in twenty-eight cases of diabetes mellitus, in some of which the condition was associated with pigmentation. The following changes were found. The hypophysis usually showed a diminution in size and weight associated with a decrease in number and with degenerative changes of the eosinophilic cells of the anterior lobe. In the thyroid more or less epithelial hyperplasia was detected. In most cases the adrenals were diminished in size and showed an associated decrease in lipid content and regressive changes in the cells of the cortex. In four cases of severe diabetes there was sclerosis of the adrenal medulla. The authors conclude that the lesions of the islands of Langerhans are fundamental in diabetes. They do not admit that the other endocrine glands play a role in the regulation of glycogen. There exist changes in the other endocrine glands of secondary importance, which however explain certain syndromes sometimes associated with diabetes, such as thyrotoxicosis, genital dystrophy, hypertension, etc.

PERRY J MELNICK

THE RETICULAR REACTIONS AND FUNCTIONS OF THE SPLENIC LYMPH FOLLICLES J WATJEN, *Centralbl f allg Path u path Anat* **62** 1, 1935

Retrogressive and progressive changes have been noted in the splenic lymph follicles after poisoning with certain karyoklastic substances. The regressive changes in the lymphatic cells were proportionate to the progressive changes in the reticulum. Questions then arose as to whether the proliferative reaction followed lymphatic damage or whether it was a primary reaction and whether this was an antitoxic reaction. Haranghy answered these questions positively from observations in diphtheria and in ricin poisoning. Watjen is concerned with corroboration of this work and in many respects is in accord with Haranghy. Changes in the lymph follicles are variable because the germinal centers have an upward and a downward phase and are more easily affected by poisons in the full development or early regression stages. Changes after ricin poisoning are apparent within

eighteen hours, and iron pigment is encountered at this period and thereafter in the reticulum cells of the follicles. This feature is important because it demonstrates activity in support of the reticulum cells of the pulp which usually exercise such a function. It is seen only after rarefaction and activation of the follicle have occurred. In protracted ricin poisoning a perinodular leukocytic wall is noted, and this speaks for the nodules acting as poison depots and exerting a chemotactic effect. The changes noted in diphtheria in human beings can be compared only tentatively with those noted in experimental ricin poisoning in guinea-pigs. In those dying of toxic diphtheria early, nuclear disintegration is found in the follicles. Those who withstand the disease longer have a stimulation of the reticulum even to the formation of epithelioid centers. These changes are indicative of the action of a weak poison.

GEORGE RUKSTINAT

DILATATION OF THE ESOPHAGUS H. ARNOLD, *Centralbl f allg Path u path Anat* 62 49, 1935

The condition forming the basis for this discussion was encountered post mortem in a woman, aged 49 years, who died of cardiac decompensation and mitral stenosis. Symptoms referable to the esophagus dated from childhood, when a feeling of fulness after eating and vomiting of food taken the previous day were noted. At the ages of 15 and 20 years sounds had been passed in the esophagus, and a condition of stenosis diagnosed. The stenosis involved the lower half of the esophagus, while the upper half was dilated. At necropsy the esophagus was 32 cm long, and its circumference varied between 8 and 10 cm. The upper third was altered by a granular esophagitis. There was an ulcer at the pylorus. Arnold believes that the dilatation described was present long before the age of 15 years and was inaugurated by cardiospasm. He emphasizes the fact that functional difficulties such as cardiospasm or achalasia are manifested earlier in life and more strongly than are such organic alterations as carcinoma of the cardia. The same observation holds true elsewhere in the gastro-intestinal tract as in pylorospasm or Hirschsprung's disease. During adolescence and early adult life compensation in the form of muscular hypertrophy was ample to overcome the stenosis. The waning response with approaching old age was further hindered by the marked mitral stenosis and periodic cardiac decompensation.

GEORGE RUKSTINAT

A BONE MARROW NODULE IN THE PARARECTAL FAT H. KUDLICH, *Centralbl f allg Path u path Anat* 62 83, 1935

In a woman, aged 49 years, who died from pneumonia a mass the size of a walnut was found in the fat between the rectum and the sacrum. On microscopic examination this proved to be composed of typical bone marrow. Kudlich briefly reviews the literature on foci of bone marrow in such locations as the adrenal glands or adrenal rests. He is certain that the sympathicoblasts or round cell collections found in some of the recorded marrow masses of the adrenal glands could play no part in the production of the pararectal growth which he encountered. He points out the observations of Petri and Gruber that the fat of human embryos in its development goes through a preliminary stage that resembles bone marrow. The growth in the present case might be regarded then as a local lack of development. The claims of Herzenberg and Patrassi that such marrow tissue might have a postfetal development from the endothelial cells of blood vessels are also cited. Either of the latter views could account for the development of bone marrow in any part of the body.

GEORGE RUKSTINAT

TOTAL CAVERNOUS TUBERCULOSIS OF THE LEFT LUNG L. ELLIOTT SILTZBACH, *Virchows Arch f path Anat* 292 652, 1934

Under the designation "left-sided total cavernous lung" Siltzbach presents from Erdheim's institute the roentgenographic and the gross and microscopic observations in nine cases of a form of pulmonary tuberculosis that has received scant attention.

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All the patients were women whose ages varied from 22 to 39 years, one was 54 years old. The clinical duration of the illness in eight patients ranged from five to twenty years, in one patient it was one year. The left lung was involved in each instance. Of nine examples of a similar condition collected from the literature, two dating back to 1803 and 1847, five occurred in women. In eight of these cases the left lung was involved. In Siltzbach's cases the left lung had been transformed into one or two large cavernous spaces bounded by dense fibrous tissue derived from the adherent visceral pleura. Remnants of parenchyma could be detected in the lower but not in the upper lobe. In the fibrous walls of the cavities there were numerous focal aggregations of lymphocytes and a few miliary tubercles. There were also many encapsulated caseous areas. The lining of the cavities was formed by a zone of nonspecific chronic inflammatory tissue with leukocytic infiltration. The fibrous pleural adhesions were rich in elastic tissue. The hilar nodes contained tubercles. The right lung revealed older encapsulated caseous foci and areas of more recent tuberculosis. In a brief supplemental note two further cases are described, which also occurred in women and involved the left lung.

O T SCHULTZ

WEIGHT OF THE THYMUS IN NEW-BORN INFANTS P R RUSSKOFF, Virchows Arch f path Anat **293** 113, 1934

The weight of the thymus, absolute and relative to body weight, was determined in 841 infants who came to necropsy in the pathologic institute of the University of Bern, Switzerland, during the years 1908 to 1932 inclusive. Premature and full-term infants who died within the first month of life were included, as were also stillborn infants. The material was divided into five groups according to body weights. In general, the weight of the thymus was slightly higher in male infants than in female infants of the same group. In a group of infants who were at least 50 cm long and who lived not longer than twenty-four hours, the average weight of the thymus was 11.8 Gm in males, and 12.5 Gm in females, and in the two sexes it was 12.1 Gm. The relation of thymus weight to body weight was 1:271.8 (0.37 per cent). The thymus was heavier in anencephalic infants than in normal infants of the same weight. In the later months of fetal life the growth of the thymus parallels that of the body. The weights of the thymus and thyroid gland did not parallel each other. The average weight of the thymus in the gouter district of Bern did not exceed that of other regions.

O T SCHULTZ

CARTILAGE RESTS IN THE TONGUE C GENTSCHIEFF, Virchows Arch f path Anat **293** 129, 1934

In carnivorous animals and in swine there occurs normally at the inferior margin of the anterior portion of the septum of the tongue a wormlike fibrous thickening that has been known as the "lyssa" or "fury worm", its name indicates that superstition ascribed to it a rôle in rabies. This structure may contain small islands of cartilage. In 1895 Nusbaum and Markowski noted the occurrence of islands of cartilage in a similar situation in the tongues of 30 per cent of new-born infants and fetuses of the eighth and ninth months examined by them, according to these authors, the cartilage disappears in later life. The accidental discovery of islands of cartilage in the tongues of two adults led Gentscheff to make further investigation of the matter. Including the two cases first observed, cartilage was found in the tongue in nine of thirty-four subjects of necropsy, three times in infants from 1 to 16 days old, once in a child 4½ years old, and five times in adults aged from 57 to 68 years. The cartilaginous islands, which may be single or multiple, were always situated approximately 1 cm from the tip of the tongue at a depth of from 1.5 to 4.5 mm from the inferior surface. Gentscheff concludes that the cartilaginous islands are phylogenetic skeletal rests of the cartilaginous rod of the reptilian tongue and that they correspond to the lyssa of the tongue of carnivorous animals.

O T SCHULTZ

THE AXIAL SKELETON IN ANENCEPHALY AND CRANIORACHISCHISIS B DEPPE,
Virchows Arch f path Anat **293** 153, 1934

Study of a series of fetuses presenting varying grades of anencephaly and craniorachischisis led Deppe to conclude that maldevelopment of the central nervous system is the primary anatomic factor, to which the failure of union of the bones of the cranium and vertebral column is secondary. Ribs and vertebral bodies are formed in normal numbers but are often fused. The characteristic distortions of the spinal column are the result of mechanical factors, such as pressure and muscular traction, to which the open vertebral column is less resistant than the closed one.

O T SCHULTZ

CHANGES IN THE CENTRAL NERVOUS SYSTEM DUE TO AN ELECTRIC CURRENT
S JELLINEK and E POLLAK, Virchows Arch f path Anat **293** 165, 1934

Death usually results so quickly after electric shock that the central nervous system reveals only slight changes. If necropsy is delayed, it may not be possible to decide to what extent the changes noted are postmortem in character. The brains of two persons who died twenty-four hours and six days, respectively, after contact with an electric current were examined microscopically. In the second case the observations were complicated by the fact that death was due to tetanus. In both cases the most striking features were engorgement of the venules and arterioles and microscopic hemorrhages. The latter were most numerous in the tissue about the ventricular system, especially about the third and fourth ventricles and the aqueduct. In the first case there was also calcium incrustation of the small vessels and capillaries of the globus pallidus as well as deposition of calcium about the vessels in the form of droplets. The localization of this process is ascribed to the course and structure of the vessels of the globus, to which the changes noted after carbon monoxide poisoning are also ascribed. The authors postulate damage to endothelium and metabolic changes in it and in the immediately surrounding tissue.

O T SCHULTZ

ARTEFACTS IN THE CENTRAL NERVOUS SYSTEM IN RELATION TO CHANGES ASCRIBED
TO AN ELECTRIC CURRENT F BOEMKE, Virchows Arch f path Anat **293** 180,
1934

Schridde has repeatedly maintained that in his large material he has never seen hemorrhages in the brain as the result of the passage of an electric current. Larger hemorrhages he ascribes to the fall which the electrically shocked person usually sustains. The minute capillary hemorrhages described by Jellinek, he maintains, are pure artefacts. In the work here reported, which was carried out in Schridde's institute, the brains of ten persons who had not died of electric shock were examined for changes previously ascribed to electric currents, these brains had been removed in the usual course of necropsies. In fifteen other necropsies the brains were somewhat more carelessly removed and were squeezed by the hands in removal. Not only in the second group of brains, but also in the first, the author claims to have observed all the alterations that others have ascribed to electric currents. Such changes are all artefacts due to the force used in removal of the brain. Minute areas in which the tissue does not take the myelin sheath stain well, described by Jellinek, are due to faulty staining technique, according to Boemke.

O T SCHULTZ

Microbiology and Parasitology

APICAL LOCALIZATION OF PULMONARY TUBERCULOSIS JEROME J HURWICH and
GEORGE MILLES, Am Rev Tuberc **31** 151, 1935

In monkeys following intravenous injection of tubercle bacilli many of the bacilli are picked up by the endothelial cells of the capillaries of the lungs. They are then ingested by macrophages and either enter the pulmonary veins to be distributed over the entire body or are excreted into the alveolar spaces. Apical

tuberculosis may be due to an excretion of tubercle bacilli from the blood stream into the alveolar spaces of the apexes, where aeration is deficient, the tubercle bacilli having reached the blood stream from the primary tuberculous complex.

H J CORPER

THE STABILITY OF THE COLONIAL MORPHOLOGY AND PATHOGENICITY OF BCG
DOROTHY M BEHNER, *Am Rev Tuberc* **31** 174, 1935

BCG (*Bacillus Calmette-Guérin*) did not acquire virulence in laboratory cultures on liquid mediums containing either normal human serum or homologous antiserum, or in the depths of nutrient broth, or by successive selection for propagation of smooth-appearing colonies on solid mediums, or by repeated passages through animals. The morphologic variation seen in the colony under different environmental conditions was not accompanied by enhanced pathogenicity. A virulent bovine strain was resistant to laboratory modification, but a slight attenuation in the virulence was produced together with a slight morphologic change in the colony, which acquired some characteristics usually associated with the rough variant. Avian strains were found to be labile, acquiring morphologic changes in colonial type in both S to R and R to S directions, and also some marked variations of each type, together with changes in pathogenicity under environmental conditions similar to those imposed on BCG strains.

H J CORPER

LESIONS IN RABBITS FOLLOWING INOCULATION WITH *BACILLUS CALMETTE-GUÉRIN*
(BCG) W H FELDMAN, *Am Rev Tuberc* **31** 323, 1935

A strain of *Bacillus Calmette-Guérin* obtained from Calmette in 1930 and subsequently grown for twenty generations on an egg glycerin medium was transferred to glycerin peptone broth and with this culture six rabbits were inoculated intravenously and four guinea-pigs subcutaneously. One of the rabbits died ten days after inoculation and the other five were killed one hundred and seventy-four days after inoculation. Numerous and striking focal lesions morphologically like tubercles were found in the lungs of each of the five rabbits. Attempts to culture acid-fast bacteria from the lesions were futile, although bacteria of this character were readily demonstrable in appropriately stained sections of the lesions. Emulsions prepared from the involved tissues in each of the five rabbits failed to produce demonstrable lesions in other rabbits or guinea-pigs, and attempts to repeat the results in later experiments failed. The results indicate that *Bacillus Calmette-Guérin* in the lungs of rabbits may at times produce numerous and extensive tubercle-like lesions.

FROM THE AUTHOR'S SUMMARY

TRICHINOSIS W W SPINK and D L AUGUSTINE, *J A M A* **104** 1801, 1935

Thirty-five sporadic cases of trichinosis occurring in and around Boston during the past three years were analyzed. The most reliable diagnostic aid in these cases was the presence of eosinophilia. The skin test usually became positive about the seventeenth day of the infection and the precipitin test usually at the end of the fourth week. These tests were of especial diagnostic aid in the early stages of the disease, when they were first negative and later became positive. Mild sporadic and chronic cases of trichinosis were often detected only by these tests. Other laboratory procedures, such as searching for the parasite in the stools, blood and spinal fluid, were time-consuming and the larvae only rarely found.

FROM THE AUTHORS' SUMMARY

THE RELATION OF BACTERIUM GRANULOSIS TO TRACHOMA F F TANG and C H CHOU, *J Infect Dis* **56** 264, 1935

Attempts to isolate *Bact. granulosis* from 179 cases of classic trachoma failed. The specimens used for cultivation consisted of lacrimal secretions, epithelial scrap-

ings, follicular contents and tarsectomized tissue. The mediums used were the semisolid "leptosira" medium of Noguchi and carbohydrate blood agar plates.

Attempts to induce trachoma in rhesus monkeys and in man by subconjunctival injection of cultures of *Bact. granulosis* failed.

A disease of the conjunctiva characterized by follicle formation but with no pannus formation or papillary hypertrophy was induced in two of nine monkeys inoculated with human trachomatous material. *Bact. granulosis* was not isolated at any time from either of these animals.

Antibodies against *Bact. granulosis* could not be demonstrated in the serums of twenty-six subjects suffering from acute or chronic trachoma.

FROM THE AUTHORS' CONCLUSIONS

THE INFLUENCE OF THE pH ON DISSOCIATION OF *BACILLUS FRIEDLANDER* AND *MYCOBACTERIUM TUBERCULOSIS* W. STEENKEN JR., *J. Infect. Dis.* **56** 273, 1935

B. Friedlander dissociates best in mediums of acid reaction. *Myco-tuberculosis-humanis* (H_{37}) becomes attenuated on acid synthetic mediums buffered with tenth-molar double potassium phosphate salts and on weakly buffered glycerol beef broth, and retains its pathogenicity on buffered alkaline synthetic medium prepared with double potassium phosphate salts. Acid glycerol potato bile medium favors attenuation of BCG. If BCG is grown on buffered synthetic medium over a period of a year or more it may regain its virulence. Stock medium weakly buffered above pH 7 tends to become acid on standing in an incubator.

FROM THE AUTHOR'S CONCLUSIONS

EXPERIMENTAL BRUCELLIASIS IN DOGS W. H. FELDMAN, J. L. BOLLMAN and C. OLSON JR., *J. Infect. Dis.* **56** 321, 1935

Two strains of *Brucella abortus* obtained from a swine and a bovine source, respectively, were introduced into eleven adult mongrel dogs to determine (1) if they would induce a definite state of disease and (2) if there was any significant difference in their pathogenic behavior. Five of the dogs received the bacteria in suspension intravenously. Six were made to fast for twenty-four hours and then were fed the organisms mixed with raw meat.

Brucella agglutinins developed as early as the fourth day in the animals receiving the organisms intravenously. In this group titers of from 1:800 to 1:1,600 were not uncommon one week after the introduction of the organisms. Successive titers varied considerably in many cases. In the animals receiving the infective agent orally the agglutinative response was much slower and never so pronounced.

In a few cases it was possible to isolate *Brucella abortus* from the blood stream but only within two or three weeks after the introduction of the infective material. From two of the dogs it was possible to recover the organism in the urine.

Although most of the dogs lived for several months after receiving the bacteria clinical symptoms of disease and specific lesions of minor significance were observed in only one dog. From only two of the dogs were the organisms recovered after death, both had received the infecting inoculum intravenously, one dog thirty-nine days and the other one hundred and eighty-five days previously.

There was no discernible difference in the pathogenic propensities of the two varieties of *Brucella abortus* used.

While the dog is capable of producing *Brucella* agglutinins following experimental introduction of either the swine or the bovine variety of *Br. abortus*, a profound resistance to the organism exists which precludes, in most instances, the development of clinical symptoms and specific lesions.

FROM THE AUTHORS' SUMMARY

EXPERIMENTS WITH THE VIRUS OF INFECTIOUS ECTROMELIA A W DOWNIE and
C A MCGAUGHEY, J Path & Bact 40 297, 1935

Growth of the virus of infectious ectromelia in tissue cultures could be inhibited for several days through the action of immune serum. Even when sufficient immune serum was present in the cultures to neutralize the virus (by *in vivo* tests) the virus was not killed. Multiplication of the virus in the presence of immune serum could always be demonstrated after from eight to twelve days' incubation. The state of the virus used as the inoculum was important in determining the effect of the immune serum. When a Berkefeld filtrate was used the inhibitory action of immune serum was readily demonstrated. With culture virus the tissue cells in the inoculum seemed to protect the virus from the immune serum and no inhibition of growth was apparent.

FROM THE AUTHORS' SUMMARY

ISOLATION OF THE VIRUS OF PLEUROPNEUMONIA F F TANG ET AL, J Path & Bact 40 391, 1935

The virus was isolated by ordinary methods and cultivated on various mediums, particularly Bennett's broth (*J Comp Path & Therap* 45 257, 1932). Five stages of development are described: elementary bodies (rings, granules and a few coccoid forms and rods), a stage in which filaments formed from elementary bodies, branching of filaments, formation of chains by the protoplasm in the filaments, disintegration of the chains into elementary bodies. No serologic differences could be made out in the different strains studied.

COMBINED CHEMOTHERAPY OF EXPERIMENTAL TRYPANOSOMA INFECTIONS C H BROWNING and R GULBRANSEN, J Path & Bact 40 425, 1935

In experimental infections with a strain of *Trypanosoma brucei* in mice it has been shown that combined therapy in which tryparsamide and a styryl compound are used in sequence produces a greater curative effect than follows the use of much larger doses of either substance alone. The evidence is strongly in favor of this result being more than a mere summation of effects. It is left undecided exactly how this "potentiation" or "synergic" action is produced, but it should be noted that tryparsamide is quickly absorbed and excreted and its trypanocidal action is fairly rapid, whereas the styryl compound is slowly absorbed and acts gradually. Accordingly the advantage of combined treatment with the pair of drugs studied may be due to the prolonged influence of the styryl compound on parasites weakened by the arsenical drug, as well as to the fact that the substances differ widely in chemical constitution and so are likely to attack the parasites at different points (receptors).

FROM THE AUTHORS' SUMMARY

TUBERCULOUS ARTERITIS W G BARNARD, J Path & Bact 40 433, 1935

A case of tuberculous inflammation of the internal carotid and coronary arteries in which there was no evidence of spread from a neighboring focus is described.

FROM THE AUTHOR'S SUMMARY

STAPHYLOCOCCIC BACTERIOPHAGES F M BURNET and D LUSH, J Path & Bact 40 455, 1935

A study has been made of the phages lysing *Staphylococcus aureus* and of the relation of these phages to those previously studied which lyse a white coccus "S F". Differences among aureus strains in normal susceptibility to phage or resulting from acquired resistance are almost entirely of a nonspecific quantitative type. Normal aureus strains that are almost completely insusceptible to a strong phage adsorb it as readily as a susceptible strain. Strains with true induced resistance fail to adsorb phage. On general characters aureus phages can be divided into "strong" and "weak". All produce small sharp-edged plaques on agar. A

method of preparing high titer filtrates of weak phages by growth on cellophane agar at 22 C is described. The aureus phages may be divided serologically into three distinct types. S F phages fall into four serologic types, one of which is common to the aureus phages. As with B coli-dysenteriae phages all staphylococcic phages of a given serologic type react uniformly with regard to photodynamic inactivation by methylthionine chloride (methylene blue), power to grow on citrated mediums and inactivation by strong urea solution. Sharp distinctions may exist between different serologic types. There are clear resemblances between one serologic group of the strong aureus phages and the commonest type of small-plaque B coli-B dysenteriae phages (serologic group II of that series) which suggest a close biologic relationship between the two groups.

FROM THE AUTHORS' SUMMARY

STUDIES OF TYPHUS P LEPINE, *Ann Inst Pasteur* **51** 290, 1933

The paper reports studies of "the existence of a murine typhus virus in the western Mediterranean Basin and its characteristics." A strain of virus producing scrotal reactions in guinea-pigs in a manner similar to that of Mexican typhus, and infecting guinea-pigs and monkeys, was found commonly in the brains of wild rats. The virus attacks man, resulting in a benign infection, very probably through fleas, which are regularly infected. The relationship to other similar exanthematous fevers is discussed.

M S MARSHALL

Tumors

METASTASIS IN SQUAMOUS CARCINOMA L W PRICE, *Am J Cancer* **22** 1, 1934

The evidence available indicates that there is no correlation between the clinical condition of the patient and the development of distant metastases. There is no constant relationship between the site of the primary tumor and the site of the distant metastases. The commonest sites for metastases in this series were lungs, thirteen cases, liver, seven cases, kidneys, five cases. Less frequently metastases were formed in other situations. From a wider consideration of the development of metastases from numerous primary tumors of various types the only consistent feature that emerges is that tumors arising in certain primary sites have a tendency to form metastases in certain tissues of predilection. There is a peculiar relationship between the site of the primary tumor and the site of the secondary deposits.

FROM THE AUTHOR'S CONCLUSIONS

MELANOTIC NEOPLASMS OF THE SKIN S W BECKER, *Am J Cancer* **22** 17, 1934

Modern study of pigment, carried out mainly by means of the silver and dopa reactions, shows that at the junction of the epidermis and dermis there are specialized cells which are capable of forming pigment. The first sign of pigment activity in the embryo is a positive dopa reaction in a branched cell in this location. This is followed by the appearance of melanin granules in the branched cells and later in the palisade basal cells. These pigment-forming cells are called "melanoblasts" in contradistinction to phagocytic dermal cells which are called "chromatophores." An increase in the number of melanoblasts at the epidermo-dermal junction results in a smooth brown nevus. In elevated nevi masses of pale-staining cells are seen in the dermis which are similar in staining properties and pigment content to the epidermal melanoblasts and are thought to be derived from the same source. The source of melanoblasts is not definitely known, but more and more workers are accepting the nervous origin. If melanoblasts are located deep in the dermis a blue nevus or mongolian spot results. The distribution here is essentially the same as in the blue skin of the ape. Pigment activity due to irradiation by ultraviolet or alpha rays consists first of prominence and branching of melanoblasts, followed by hyperpigmentation of palisade basal cells. Pigment activity occurring spontaneously with no demonstrable cause results in the same

histologic picture, and the lesion is known as "lentigo," which has nothing to do with the common freckle known as "ephelis." If this stimulation of pigment activity increases to the point of melanoblastic proliferation, the lesion is known as "lentigo maligna" and is already malignant melanoma. Further activity results in melanoma of the fusiform cell type—the so-called melanosarcoma—or of the ovoid cell type known as "melanocarcinoma." The occurrence of both types of cell in the same primary or metastatic growth demonstrates the futility of trying to classify them as sarcoma or carcinoma, the best designation being "malignant melanoma." Melanoma arising from a pigmented nevus has its origin in the melanoblastic cells at the epidermodermal junction and not in deeply lying nevus cells as has been sometimes supposed. "Melanotic epithelium" and "pigmented epithelioma" are terms used to designate a benign epidermal neoplasm containing considerable melanin. These lesions are closely related to the so-called senile or seborrheic verruca and almost never undergo malignant degeneration. Study of cutaneous carcinomas showed that 33 per cent of the basal cell tumors, 14 per cent of the intermediate, 9 per cent of the mixed and 7 per cent of the squamous cell tumors contained melanin demonstrable by the silver technic. The pigment in these tumors is due to the presence of melanoblasts which cannot be distinguished from normal melanoblastic cells as regards type and arrangement of melanin granules. In rather unusual cases carcinoma of the breast which has invaded the skin is intimately associated with melanoblastic cells, which are also normal.

FROM THE AUTHOR'S SUMMARY

EWING'S SARCOMA C. L. CONNOR, *Am J Cancer* **22** 41, 1934

From the three cases recorded here I believe one may say that an endothelioma of bone is much like a malignant endothelioma elsewhere, and that it does not respond readily to treatment with x-rays but disappears gradually and slowly as compared, for instance, with a metastatic lymphosarcoma or a neuroblastoma in bone, but at the same time much more rapidly and surely than an osteogenic sarcoma or the average metastatic carcinoma. Since my previous report I have discovered that metastatic lymphomatous tumors are the most difficult to differentiate from endothelial myelomas. The former may manifest themselves first as bone tumors, and it may not be until generalized lymphadenopathy in superficial areas occurs that the question of a lymphoma is raised. Perhaps there is more evidence that this tumor differs from a lymphosarcoma and other hematocytoblastic tumors in the fact that metastases to the lymph nodes were not present in any of these cases. Ewing's sarcomas constantly metastasize to the lungs, behaving in this respect like connective tissue sarcomas, in contrast to lymphosarcomas and myelomas. It may be found on further study that metastases to the lymph nodes are not common, and an opinion to the contrary, previously expressed, may have to be revised. Another revision can be made with the evidence at hand. The tumor cell of Ewing's sarcoma can become an osteoblast and form bone.

AUTHOR'S SUMMARY

THE UTILIZATION OF SIMPLE DERIVATIVES OF GLUCOSE BY MOUSE SARCOMA O. O. MEYER, C. McTIERNAN and W. T. SALTER, *Am J Cancer* **22** 76, 1934

A study of the glycolytic breakdown of various carbohydrates by tumor tissue shows the same sugars glycolyzed as are utilized by normal tissue. The anomalous aspect of the carbohydrate metabolism of malignant tissue springs from a difference in degree rather than in kind.

FROM THE AUTHOR'S CONCLUSIONS

AMMONIA PRODUCTION BY SARCOMA. THE SPARING EFFECT OF THE CARBOHYDRATE W. T. SALTER and P. D. ROBB, *Am J Cancer* **22** 87, 1934

The utilization of various carbohydrate derivatives by mouse sarcoma 180 has been studied with respect to the lessening production of ammonia by the malignant tissue. The carbohydrates with which there is the most decrease in the production

of ammonia by sarcoma are those which are best glycolyzed That liver shows no such effect is consistent with its low glycolytic index Cyanide affects neither glycolysis nor the sparing of ammonia, but iodo-acetate checks both The total of nonprotein nitrogen eliminated is unaffected by the presence of sugars which spare ammonia

FROM THE AUTHORS' CONCLUSIONS

ARSENICAL KERATOSIS AND CARCINOMA C C FRANSEEN and G W TAYLOR, *Am J Cancer* **22** 287, 1934

Nine cases of carcinoma due to arsenic and five cases probably of arsenical origin are reported Arsenic deposited in the skin may manifest its carcinogenic property as late as forty years after the ingestion of, or occupational exposure to, arsenic The carcinogenic property of inorganic arsenic is not universally appreciated, and, as a result, carcinoma of the skin may inadvertently be produced Inorganic trivalent arsenic, usually in the form of Fowler's solution (solution of potassium arsenite), appears to be the chief carcinogenic agent Chronic arsenical lesions following the administration of organic arsenical compounds are exceedingly rare Arsenical carcinomas are not invariably of the squamous cell type, as evidenced by the fact that more than one third of the carcinomas in this series were of the basal cell type Although the malignancy of the squamous cell lesions is usually of low grade, metastasis to the groin and axilla is not infrequent, as attested in our series in nine lesions, two thirds of which were graded 1 histologically With all lesions of considerable size, therefore, the regional lymph nodes should be removed, in spite of low histologic grading Patients with early arsenical lesions may be spared extensive operations or untimely death by prophylactic destruction of precancerous arsenical keratoses, or, by careful and frequent observation, these keratoses may be destroyed at the moment malignant changes threaten

FROM THE AUTHORS' CONCLUSIONS

A NEOPLASTIC DISEASE OF THE KIDNEY OF THE FROG B LUCKE, *Am J Cancer* **22** 326, 1934

Three of 276 leopard frogs with neoplastic disease of the kidney had tumors in organs distant from the kidneys one, in the liver and bladder, a second, in the liver and left orbit, and a third in the liver These tumors are believed to represent true metastases The occurrence of such metastasis in cold-blooded vertebrates generally is discussed

FROM THE AUTHOR'S SUMMARY

ROFFO'S TEST IN CANCER STATISTICAL RESULTS OF 11,000 CASES A GANDOLFO, *Am J Cancer* **22** 363, 1934

Though Roffo's test is not specific, it is of value as an auxiliary method in the diagnosis of cancer The technic is as follows To 1 cc of fresh, clear serum, 5 drops of 1 per cent neutral red in distilled water are added If the serum assumes a red color, the reaction is regarded as positive A yellowish hue indicates a negative reaction The following precautions are to be taken 1 The serum is not to be obtained by centrifugation, the blood must coagulate and the limpid serum be extracted by means of a suction pipet, the slightest trace of hemolysis altering the results 2 The blood must be withdrawn before breakfast, in order to avoid the influence of nutritive lipoids 3 Neutral glass tubes must be used, as alkalinity influences the results

A negative test does not exclude the presence of a tumor, a positive test should induce one to continue investigations, in order to discover the tumor, as the proportion of erroneous positive results is small (6.37 per cent in 6,718 cases) The test has given a high percentage of positive results in cancer of the uterus (68.33 per cent), ovary (78.94 per cent), bladder (72.41 per cent), stomach (76.26 per cent), intestine (76 per cent), liver (84.20 per cent), pancreas (84.61 per cent), lungs (75 per cent) and mediastinum (86.66 per cent), and in osteosarcoma (71.42 per cent), all of which generally offer greater difficulties in clinical diagnosis

FROM THE AUTHOR'S SUMMARY

BENIGN NEOPLASMS OF THE RAT'S BREAST J HIFMAN, *Am J Cancer* **22** 497, 1934

Benign fibroma and fibro-adenoma of the rat breast are easily and continuously transplantable. They grow, when transplanted, not only in the region of the mammary glands, but also in the axillae, groins, nape of the neck and outer side of the thigh and in the abdominal cavity. The transplanted tumors do not always retain the structure of the spontaneous tumors from which they are derived. The transplantability of a tumor is not a criterion of malignancy. Although the growth energy of these tumors fluctuates widely, there has been no cessation in one series for fifty-three generations, and in a fourth series for sixteen generations, during a period of ten years. They grow as readily in adult rats as in young rats. In the former the growth tends toward glandular hyperplasia, in the latter toward a marked increase of fibroblasts. Three of the six primary fibro-adenomas of the breast which were transplanted through four generations or more became actively growing cellular tumors with the morphology of sarcoma. Some of the tumors ulcerate through the skin, but this is due only to pressure on the skin and is not an evidence of malignancy. With a large number of inoculations and the implantation of two or more fragments 3 mm in diameter, these benign tumors are readily transplantable for many generations in suitable hosts. The benign tumors as they develop into sarcoma require smaller and fewer fragments for transplantation. Dr Otto F Krehbiel has transplanted one such tumor by the trocar method, using 3 mg of tumor substance, for fifty-six generations. Of sixteen rats with spontaneous benign tumors, six (37 per cent) yielded tumors transplantable for from four to fifty-three generations.

FROM THE AUTHOR'S SUMMARY

TERATOMA OF TESTIS C C HERGER and A A THIBAUDEAU, *Am J Cancer* **22** 525, 1934

The embryonal carcinomas with lymphoid stroma proved the most radiosensitive and yielded the most satisfactory clinical results. Experience with the quantitative Aschheim-Zondek reaction convinces us of its value as a diagnostic procedure and as an aid in the conduct of treatment.

FROM THE AUTHORS' SUMMARY

THE CARBOHYDRATE TOLERANCE IN CANCER AND THE EFFECT OF ROENTGEN RADIATION F H L TAYLOR and H JACKSON JR, *Am J Cancer* **22** 536, 1934

In only one third of thirty-five patients with cancer was a decreased tolerance for dextrose found prior to the institution of roentgen therapy. Consideration must be given to the fact that a lowered tolerance for dextrose is not an uncommon tendency in persons between 55 and 70 years of age, particularly if they are suffering from malnutrition. The presence of a lowered tolerance for carbohydrate in patients with cancer is not diagnostic nor is it of assistance in prognosis. However, a progressively increased abnormality of the tolerance for carbohydrate may be regarded as an unfavorable sign. Roentgen radiation has no consistent effect on the sugar tolerance of cancerous patients. No relationship has been found between the decreased tolerance for sugar occurring in some patients with cancer and the total serum calcium.

FROM THE AUTHORS' SUMMARY

ROUND-CELL, SPINDLE-CELL, AND NEUROGENIC SARCOMAS OF THE LIP T DE CHOLNOKY, *Am J Cancer* **22** 548, 1934

In the available literature reports of 20 cases of sarcoma of the lip were found, in only 5 of which complete records were given. Four additional cases are reported: one of round cell sarcoma of the upper lip, two of spindle cell sarcoma and one of neurogenic sarcoma of the lower lip. In all cases the growth was on the vermillion border. In three of the cases the sarcoma was treated by wide

local excision followed by dissection of the regional nodes The tumor on the upper lip was removed by electrocoagulation The patients were all symptom-free after periods of six months (the one with round cell and one of the two with spindle cell sarcoma), eight months (the one with neurogenic sarcoma), and four years (one with spindle cell sarcoma)

FROM THE AUTHOR'S SUMMARY

METABOLIC STUDIES IN MOUSE LEUKEMIA J VICTOR and M R. WINTERSTEINER, Am J Cancer **22** 561, 1934

The oxygen consumption, aerobic glycolysis and anaerobic glycolysis of lymph nodes of normal mice and of those inoculated with different lines of transmissible lymphatic leukemia were measured in animals in which age and genetic constitution were controlled There are metabolic differences between normal and leukemic lymph nodes Inherent metabolic differences were observed not only between different lines of leukemia derived from the same organ in different spontaneous cases, but also between lines derived from different organs in the same spontaneous case The metabolism of the individual line of transmissible leukemia was consistent during the period of observation In no case was the oxygen consumption of leukemic nodes less than normal The anaerobic glycolysis was always greater than normal The results are statistically compared with others reported in the literature Factors causing variations in results are discussed

FROM THE AUTHORS' SUMMARY

THE BEARING OF GENETIC WORK WITH TRANSPLANTED TUMORS ON THE GENETICS OF SPONTANEOUS TUMORS IN MICE C C LITTLE, Am J Cancer **22** 578, 1934

"The skepticism and criticism of genetic work by non-geneticists is no new development in the broad field of cancer research Similar attitudes might be cited on the part of surgeons for radiologists, on the part of chemists for pathologists, and vice versa The point seems to be that, as our knowledge of the complexity of cancer increases, greater intolerance by various specialized groups and individuals, for the work of specialists of other types, is needed In the fight against cancer, experimental genetics certainly has its place In the further development of cancer research, in all three levels of transplantable, induced, and spontaneous tumors, the genetic point of view will eliminate or analyze variables and will continue to make for accuracy and predictability of results"

THE DEVELOPMENT OF MULTIPLE TUMORS IN TARRIED AND RADIATED MICE M C REINHARD, A A THIBAudeau and C F CANDEE, Am J Cancer **22** 590, 1934

As a result of one experiment involving the use of more than one hundred mice there is no evidence that short wavelength radiation changes the susceptibility of mice to the production of tar tumors, nor does the radiation alter the carcinogenic power of the tar used in this experiment We believe that the low incidence of spontaneous tumors in the nonirradiated mice is a direct result of the tarring In the irradiated group the low incidence may be attributed to the tarring also, or to the irradiation, or to a combination of both Of course, it is possible that the occurrence of the spontaneous tumors has merely been retarded, and, had the mice lived longer, a percentage of mammary carcinomas might have been obtained which would more nearly approximate the normal expectancy The appearance of the multiple tumors, distant in all cases from the site of painting, may be considered as evidence in favor of a general action of the tar This is especially emphasized by the very striking absence of tumors at the site of painting The possibility of chance contact must not be overlooked, but in view of the failure to produce tumors at the site of painting itself, this possibility is somewhat remote On the other hand, we again point to the difference in the histologic picture as between the multiple sebaceous adenoma produced in this series and the typical tar cancer produced locally by repeated paintings with this agent

FROM THE AUTHORS' SUMMARY

A STUDY OF THE SERUM OF CHICKENS RESISTANT TO ROUS SARCOMA F G BANTING and S GAIRNS, *Am J Cancer* **22** 611, 1934

Serum resistant to Rous sarcoma neutralizes extract of Rous tumor, this neutralization occurs during incubation. The neutralizing power is destroyed between 70 and 80 C. Intravenous administration of the serum has no effect on a growing Rous tumor. Serum resistant to Fujinami sarcoma does not neutralize Rous tumor extract. Serum resistant to Rous sarcoma does not neutralize Fujinami extract.

FROM THE AUTHORS' SUMMARY

ROUS SARCOMA TISSUE GRAFTS IN SUSCEPTIBLE AND RESISTANT CHICKENS D IRWIN, S GAIRNS and F G BANTING, *Am J Cancer* **22** 615, 1934

In both susceptible and resistant birds all the tumor cells of Rous sarcoma sac grafts became necrotic. In susceptible birds the host cells surrounding the sac grafts gave rise to fatal tumors. In resistant birds small amounts of tumor-like tissue formed adjacent to the sac graft, this tissue failed to take on malignant characteristics and subsequently regressed. Cells and the tumor-producing substance in the sac grafts did not survive more than forty-eight hours in resistant birds.

FROM THE AUTHORS' SUMMARY

NEPHROGENIC TUMORS C F GESCHICKTER and H WIDENHORN, *Am J Cancer* **22** 620, 1934

In spite of the difficulty in demonstrating the remains of embryonic nephrogenic tissue in the more slowly growing malignant nephromas, and in the nephrogenic zone of the opposite kidney, comparisons of the Wilms tumor, the benign and malignant cystadenomas and the more rapidly growing malignant nephromas in young adults indicate that all of these nephrogenic tumors have their origin in the same mother substance. Judging from the microscopic studies, this tissue of origin is the renal blastema, a compact growth of spindle cells which differentiate into small tubular structures composed of oval epithelial-like cells. This type of primitive tissue predominates in the Wilms tumors in children, the spindle cells being more prominent than early tubule formation. In many of the benign and malignant cystadenomas of young adults, spindle cells and small tubules composed of oval epithelial cells predominate, particularly in those growths which have undergone malignant change. In the nephromas, occurring in old age, the blastemal elements are difficult to demonstrate, except in the more malignant and rapidly growing ones, but nevertheless they do occur. Taking the three groups of tumors as a whole, the shift from immature spindle to adult epithelial forms appears to represent the cycle of growth within the individual tumor, and the extent of maturity reached, as shown microscopically by scarcity or prevalence of larger epithelial elements, can be correlated definitely with the age incidence of the tumor and its relative degree of malignancy. The fact that all of these neoplasms show a distinct tendency to make their appearance just beneath the capsule in the normal growth zone of the kidney, and the fact that the cortical zone of renal tissue normally continues to grow until adult life, relate all of the growths to a single type of developing tissue. Hence the present study seems to indicate that the variations in structure which make for separate types of tumor are the expression of the various rates of growth and the extent of differentiation achieved by the individual form of tumor, rather than of their origin from separate and distinct tissues.

FROM THE AUTHORS' SUMMARY

CARCINOID TUMORS OF THE SMALL INTESTINE E M HUMPHREYS, *Am J Cancer* **22** 765, 1934

In a series of 3,200 cases in which autopsy was performed there were 3 of carcinoid tumor with metastases in regional lymph nodes. In 2 of these the carcinoids were multiple, one with 2, the other with 9 independent tumors. In 2

cases the lumen of the bowel was narrowed appreciably, and while the clinical evidence, though suggestive, was not conclusive, the anatomic features indicated a low grade intestinal obstruction. In the same series there were 5 cases of solitary carcinoid tumor without metastases, 1 in the jejunum, 3 in the ileum and 1 in the rectum (a carcinoid polyp). Thus in this series there was an incidence for all carcinoids of 0.25 per cent, for carcinoids of the small intestine, 0.22 per cent, for multiple carcinoids, 0.06 per cent, and for metastasizing carcinoids, 0.09 per cent. From a study of these cases and of case reports in the literature, it is evident that the carcinoid tumor of the small intestine is a far from harmless lesion. Of 152 tumors, 24.4 per cent had metastasized, and 24 per cent were responsible for symptoms of intestinal obstruction. The incidence of multiple carcinoids in the latter series was over 30 per cent.

FROM THE AUTHOR'S SUMMARY

THE RELATIONS OF TISSUE METABOLISM TO CONSTITUTIONAL PREDISPOSITION TO CANCER B. WALTHARD, *Ztschr f Krebsforsch* 40 447, 1934

Walthard has observed, as have others, that in degenerating tissues there is frequently a diminution of oxidative activity accompanied by an increase of aerobic glycolysis. As not nearly all changes of this sort are antecedent to cancer, he is inclined to question Fischer-Wasels' identification of this type of change with the appearance of the cancerous predisposition. In his opinion, the nature of this predisposition cannot be identified so simply, and still remains unknown.

H. E. EGGERS

THE LONG-TIME TREND OF CANCER STATISTICS S. PELLER, *Ztschr f Krebsforsch* 40 465, 1934

Peller was among the first to suggest that cancer statistics, properly evaluated, show a diminution of the disease. Here he summarizes the results of such an evaluation of statistics from world-wide sources and reaches the conclusion that during the last twenty-five years there has been a real diminution in the frequency of cancer. This has been accompanied by changes in the relative frequency of primary sites, some of these showing a real increase. For males, he has no explanation to suggest for this shift, but for females he believes that diminished child-bearing may be responsible. The general diminution cannot be explained by improved therapeutics, since it occurs very strikingly in such inaccessible cancers as those of the stomach and esophagus. The statistics of Vienna for the last few years indicate that here there has been a reversal of this trend, with an actual increase there of mortality from cancer, but even so, the actual (corrected) rate there is smaller than it was from twenty to thirty years ago.

H. E. EGGERS

THE SO-CALLED PERIPHERAL REACTION OF TUMORS AND THEIR METASTASES J. R. M. INNES, *Ztschr f Krebsforsch* 40 527, 1934

A very considerable series of malignant tumors, both primary and metastatic, were studied with a view to the reaction of the immediately surrounding tissues. The picture was in all cases essentially the same, consisting of lymphocytic infiltration along with that of plasma cells and more or less connective tissue overgrowth. Special features of reaction such as are occasionally seen with implanted tumors in animals Innes regards as being responses to the presence of material actually foreign to the new host. He could also detect no relationship of this peripheral reaction to prognosis.

H. E. EGGERS

BASAL CELL CARCINOMA WITH CYST FORMATION K. ENGLMANN, *Ztschr f Krebsforsch* 40 546, 1934

Englmann reports six cases of basal cell carcinoma with the unusual feature of small cysts filled with mucus in the infiltrated epithelium. Five were primary on the face, the sixth was anal. Englmann believes that the cysts are not the

result of epithelial degeneration, but of cellular secretory activity. Unlike the usual rodent ulcers, these occur occasionally at early ages, two of the patients were aged 22 and 32 years, respectively. Ulceration of these tumors is absent or late, the tumor progressing as a deep-seated thickening of the skin. They run a slow course with indefinite infiltration, and may give late metastases.

H E EGGERS

Technical

BLOOD WASSERMANN TEST IN NEUROSYPHILIS W C MENNINGER and L BROMBERG, *J Lab & Clin Med* 20 698, 1935

In approximately 30 per cent of cases of active neurosyphilis in which tests of the spinal fluid are positive the Wassermann reaction of the blood fails to give any indication of this process. In 23 per cent neither the Kahn nor the Wassermann test of the blood is positive. In view of this fact, syphilis, after the early stage, cannot in any case be regarded as completely studied, accurately diagnosed or correctly treated without knowledge of the cerebrospinal fluid.

FROM THE AUTHORS' CONCLUSIONS

THE CALIBRATION OF GRADED COLLODION MEMBRANES W J ELFORD and J D FERRY, *Brit J Exper Path* 16 1, 1935

Methods are described for calibrating graded collodion membranes by measurements of their thickness, their specific water content and the rate of flow of water through them and by calculation from these data of the average pore diameter. The several assumptions involved have been critically examined and evidence provided for their justification when applied to membranes of porosities greater than 10 microns. The relation of membrane structure and porosity is discussed, and a possible mechanism responsible for the gradation in the porosities of nitrocellulose films is advanced.

FROM THE AUTHORS' SUMMARY

MECHANISING THE VIABLE COUNT J A REYNIERS, *J Path & Bact* 40 437, 1935

An effort has been made to mechanize the counting of viable bacteria. The thesis has been advanced that standardization of results can best be obtained by mechanizing the technic. To establish this thesis Reyniers has applied mechanical principles to what is now considered standard technic, i.e., the agar plate method, so that samples of cultures are treated in a uniform manner. The mechanized technic, called for convenience the R method, consists essentially of a mechanical distribution of bacteria on one plane and in a definite pattern on an agar surface. A comparison has been made between the R method, the standard method and the method of Wilson (roll tubes). The R method exhibits a lower percental mean deviation than either of the others. This would seem to indicate that a greater degree of standardization is possible through the use of mechanical principles which limit the technical errors and provide a uniform growing surface. The amount of growth obtained on an R plate is higher than is obtained with the standard or roll tube method. By comparison with other methods the R plate is easier to count because of the mechanical distribution of the colonies, and it permits the use of a mechanical counting device attached to a photo-electric cell. Considering the entire procedure of the R, standard and roll tube methods, the R method is easier to use and permits considerable saving of time. It is practical in field work because it allows the use of prepared agar disks, and can also be used in pure culture work.

FROM THE AUTHOR'S SUMMARY

Society Transactions

PHILADELPHIA PATHOLOGICAL SOCIETY

March 14, 1935

MORTON McCUTCHEON, *President, in the Chair*

AN UNUSUAL CASE OF CHRONIC TRACHEITIS AND BRONCHITIS D R COMAN

The case is that of a girl $2\frac{1}{2}$ years of age who came to autopsy at the Hospital of the University of Pennsylvania. She was the seventeenth child of the mother. It was noticed early that she had dyspneic and wheezing respiration, especially when nursing. This difficulty increased and became particularly obvious when the child began to walk. When about 20 months old she caught cold and was ill for two weeks with fever and occasional cough which persisted and became worse. Four months later the child had real difficulty in getting breath, and at 27 months she was dyspneic even at rest. These spells came on three or four times a day and lasted for about an hour, during which the breathing was extremely labored. The condition was progressive.

At the time of admission to the hospital the child had paroxysmal coughing and wheezing musical râles on expiration, and retraction of the intercostal spaces and supraclavicular fossae on inspiration. Expiration was prolonged and the breath sound harsh. Her temperature rose from 98 to 101 F. A roentgenogram of the chest revealed considerable prominence of the lung markings and hili. The diagnosis was "high obstruction, cause uncertain." Bronchoscopic examination revealed granulation tissue which bled easily in the trachea and bronchi. Bacteriologic examinations gave negative results. The child's respiratory difficulty increased, and she died on Nov 2, 1934.

At autopsy the mucosal surface of the trachea and bronchi presented a reddened, finely granular appearance with an overlying thin mantle of mucopurulent exudate. This lesion extended from the larynx through the trachea and along the bronchi as far as could be traced grossly. The lumens of the component parts of the respiratory tract were notably narrowed, and those of some of the bronchi were nearly occluded. Remarkable on gross examination was a peculiar increase in the cartilaginous elements of the bronchial walls. The cartilaginous plates were thicker and apparently extended farther out along the bronchial ramifications than normal. The lungs presented no other gross lesions.

The liver displayed on the broad anterior surface a few oat-sized yellowish-gray nodules. A few similar tiny nodules were found lying deep in the liver substance. The contents of these focal lesions were soft, almost caseous.

The rest of the body presented no significant departure from the normal.

The diagnosis at the time of autopsy was exudative and productive tracheitis and bronchitis and chronic focal inflammation in the liver. The peculiar increase in the cartilaginous elements of the respiratory tree suggested a congenital anomaly rather than an acquired lesion, and the clinical history of early respiratory difficulty seemed to substantiate this view.

Microscopic preparations of the trachea showed the greater portion of the mucosal surface replaced by a heavy layer of granulation tissue composed of budding capillaries and proliferating fibrous connective tissue. There was a cellular exudate composed mostly of monocytes and polymorphonuclears. Beneath this layer remnants of ductal and glandular structures were seen.

Sections of the lungs showed the larger bronchi to be the seat of a chronic inflammatory reaction similar to that seen in the trachea. The most striking feature was the markedly thickened cartilage of the bronchi. The surrounding

lung tissue showed patchy atelectasis and emphysema, and many of the smaller bronchi and bronchioles showed a peripheral aggregation of lymphocytes, monocytes and plasma cells, while the mucosa remained essentially unaltered

Microscopic section of the liver showed well circumscribed collections of exudative, cellular and proliferative inflammatory reaction not dissimilar from the reaction in the trachea and bronchi. The central portions of these foci contained densely packed cells of the large monocytic and plasma cell type surrounded by a zone of granulomatous reaction

Stains for acid-fast organisms revealed none

As regards a diagnostic classification of these lesions, much remains in the realm of speculation

Aschoff (*Verhandl d deutsch path Gesellsch* 14 125, 1910) speaks of "tracheopathia chondro-osteoplastica," which he concludes is due to disease of the elastic bands of the trachea in which cartilage and bone are developed. He cited two cases, both in elderly men. In discussion of this, Steinberg stated that two forms of this lesion occur—one which Aschoff has described and a chronic inflammatory lesion. Chiari favored the existence of three types, and Schmorl concurred in this assumption: (1) a process secondary to chronic inflammatory lesions, (2) noninflammatory ecchondroses and (3) idiopathic noninflammatory new bone formation in the mucosa and submucosa

The descriptions of cases by these and other men seem to refer more to an ecchondrosis than to a hypertrophy of the cartilage in its normal site, and their cases are apparently not of a character analogous to the case presented here, which, from its clinical history and from the results of gross and microscopic examination, suggests an underlying congenital hyperplasia of the normally situated cartilaginous plates with a secondary chronic inflammatory lesion on the basis of this malformation

MINOR HEMAGGLUTININS WILLIAM P. BELK

The blood of a young man convalescing from acute infectious mononucleosis was found to contain (in addition to an iso-agglutinin of high titer) an auto-agglutinin, four specific hetero-agglutinins and heterohemolysins for the erythrocytes of the horse, sheep, rabbit and guinea-pig, as well as the rouleau-forming property

The various characteristics of these several substances are described in detail

The appearance of so many antibodies, including the heterophilic, in the same blood suggests the action of a nonspecific stimulus rather than that of the Forssman antigen

It is suggested that infectious mononucleosis may be characterized not so much by the presence of heterophilic sheep agglutinins as by the appearance of a variety of antibodies

DEGENERATIVE CHANGES IN BLOOD LEUKOCYTES MAX M. STRUMIA

Although in recent years the morphologic examination of blood has made great strides, especially through the more general use of the nuclear shift of the neutrophils, a great wealth of information is still generally ignored. In all toxemias certain degenerative changes take place in the circulating blood cells. In the neutrophils they are particularly constant and easily determined. For practical purposes, these lesions may be divided into alterations of the granules, alterations of the cytoplasm and nuclear changes. The most obvious alterations of the granules are increase in size accompanied by hyperchromatism and polychromatophilia. Later on, when cytoplasmic and nuclear changes occur, the granules become coarse, larger, polychromatic, and finally disappear. In the cytoplasm there is edema with progressive separation of colloids leading to formation of bald patches and vacuoles. In the nucleus there is coagulation necrosis followed by swelling. The changes in the granules referred to have been pointed

but more often under the term "toxic granules" However, early changes in the granules, hyperchromatism and polychromatophilia, may occur independent of any other lesion in the cell and seem to be independent of more serious degenerative changes, such as fusion, swelling, basophilia, etc The rapid appearance or increase of the simple hyperchromatism and polychromatophilia of the neutrophilic granules and their rapid disappearance point to a reversible process Until the nature of these changes is more definitely known, it seems desirable to use a more general and distinctive term

Experimental work shows that the appearance of hyperchromatism and polychromatophilia in the granules is the earliest and most constant sign of toxemia, being present even in very mild types These changes are best studied by hourly observations of the blood in patients who have received small doses of vaccine, especially typhoid vaccine, intravenously These are followed by changes in the cytoplasm and nuclear degeneration, which is to be considered as an irreversible process indicating practically the death of the cell

INFLAMMATION AND BACTERIAL INVASIVENESS VALY MENKIN

Powerfully necrotizing irritants produce, as a result of an increase in capillary permeability and of lymphatic damage, an extremely prompt reaction, perhaps best termed "fixation" By this process the area of injury is mechanically circumscribed and the dissemination of the irritant is prevented *Staphylococcus aureus* is such a bacterial irritant *Aleuronat* is a chemical irritant of similar potency Mild irritants, on the other hand, produce only a delayed reaction, thus allowing relatively free penetration of the irritant into the circulation for a considerable interval of time In such instances occlusion of the draining lymphatics often takes place as late as two days subsequent to the inoculation of the irritant This type of irritant is exemplified by the hemolytic streptococcus Another instance has been recently demonstrated by McMaster and Hudack, who showed that up to forty-eight hours following a mere incision of the skin or local burn lymph drainage was adequate Subsequently the lymphatics failed to convey effectively the materials contained in them The intensity of fixation is found frequently to be parallel to the extent of the inflammatory edema This suggests that the local swelling is at least in part the result of blockage to the normal lymphatic drainage, which is thus unable to cope adequately with the excess outpouring of plasma from the capillaries at the site of inflammation

In relatively large suppurating or acutely inflamed areas the reaction of fixation may occur as early as thirty minutes after the injection of an irritant This prompt response allows a definite interval of time for the relatively sluggish leukocytes to assemble at the site of inflammation for the purpose of phagocytosis The neutrophils appear first, to be displaced subsequently by the macrophages This cytologic sequence, as recently demonstrated, is evidently conditioned by changes in the local hydrogen ion concentration With the development of local acidosis at the site of acute inflammation predominance in the exudate shifts from the neutrophil to the mononuclear phagocytic type It is conceivable that the mechanism of suppuration is closely associated, perhaps through an activation of autolytic tissue enzymes, with the local increase in the hydrogen ion concentration of an inflamed area Studies are now in progress in an attempt to clarify this problem

The reaction of fixation, by mechanically circumscribing the irritant in the earliest phase of the acute inflammatory reaction, plays a definite rôle in immunity, for it protects the organism as a whole at the expense of local injury The reason for the disastrous effects resulting from untimely surgical intervention with such an effective inflammatory barrier as described and as encountered, for example, in the *staphylococcus* boil or the anthrax carbuncle is quite obvious and needs no particular emphasis in view of the foregoing discussion

ARCHIVES OF PATHOLOGY
CHICAGO PATHOLOGICAL SOCIETY

April 8, 1935

I PILOT, *Presiding*

EDWIN F HIRSCH, *Secretary*

NECROSIS OF THE STOMACH FOLLOWING INTRAVENOUS INJECTIONS OF NEOARSPHEN-
AMINE O O CHRISTIANSON

This article will be published in full in the ARCHIVES OF PATHOLOGY

DISCUSSION

P R CANNON Do you think that arsenic entering the stomach in the regurgitated bile caused the changes in the gastric blood vessels?

V LEVINE Could the changes in the stomach have been due to the agranulocytosis? With that disease necrosis occurs in various tissues of the body

O O CHRISTIANSON The injury to the blood vessels probably was caused by arsenic excreted from the blood Necrosis of the lining of the stomach does not occur, so far as I know, with agranulocytosis

VALUE OF CHEMICAL AND PATHOLOGIC OBSERVATIONS IN PNEUMONOCONIOSIS, WITH
SPECIAL EMPHASIS ON SILICOSIS AND SIIICOTUBERCULOSIS HENRY C
SWEANY, JESSE E DOUGLASS and JULIUS PORSCHE

The antemortem and postmortem observations in forty-four patients most of whom had some form of pneumoconiosis were correlated with the contents of total silica in the various parts of their respiratory tracts With certain exceptions the total silica content varies roughly with the time and the type of exposure to silica When there has been exposure to coarsely divided silica or silicates (sand or dust) in people having faulty physiology of the bronchial tract (bronchiectasis, emphysema, tuberculosis, etc) the silica tends to be retained and produces changes in proportion to the amount of dust retained Petrographic analysis determines the size and quality of such particles

The silica content in people without silicosis usually varies from less than 0.5 mg per gram of dried lung in the infant to between 1.5 and 2 mg per gram of dried lung in the aged The silica content of the lymph nodes of nonsilicotic persons rises from near zero in infancy to 1 mg at 8 years of age and to 6 mg in old age In silicosis the silica content of the lymph nodes does not rise much above the amount found in nonsilicotic adults, because the nodes in the latter seem to be near the saturation point for the development of silicosis This may help to explain the early appearance of specific whorls in the lymph nodes When in people exposed to pure silica the silica content of the lung exceeds 2 mg per gram of dried lung specific nodulation may be expected to develop The silica content of the lung then rises roughly in proportion to the time and the amount of exposure to exceed the content found in the lymph nodes This indicates that the lymph nodes soon become saturated, after which they take up silica only in diminishing amounts

The silica content of the lung tissues may increase to 25 mg per gram of dried lung or more, depending on the type of work and the interval of time in the work The lungs of stone cutters, stone carvers and others exposed to such large quantities of silica have the highest The silica content of coal miners' lungs may become double the high normal limit without showing specific nodulation This may be due to the presence of silicates or, more likely, to the dampening effect of the carbon on the fibrosis When a coal miner works in silica the lymphatics seem to be blocked and show no increase in silica content, while in the parenchyma it rises to high levels This indicates not only blockage of the

lymphatics but also impairment of the bronchial and ciliary action. There is a moderate increase of the silica content of the pigmented portions over that of the nonpigmented portions. This indicates that the black portions have accumulated silica as well as carbon.

Probably the irritation due to tuberculosis causes an influx of silica-laden phagocytes from without. Any new growth or acute inflammation developing in such a lung will dilute the silica in proportion to the new cells introduced. Tuberculosis causes the development of nodules or masses that may be described as a tuberculosilicosis complex. A gross pathologic diagnosis of the disease silicosis is always possible, although nodules of silicotuberculosis may be mistaken for those of tuberculosis. Tuberculosis changes silicosis to a grave and fatal disease, sometimes terminating quickly in caseous pneumonia. Coal dust seems to retard silicosis, tuberculosis and silicotuberculosis, bringing about the development of large black masses, larger and more benign than the tuberculosilicotic complex.

The most important question yet to answer is how much the minimal silicotic nodulation predisposes to the tuberculous infection. Would these patients have died of tuberculosis (as many do) without the silicosis, or does the silicotic nodulation accentuate the tuberculosis? If we are to judge by the more advanced cases of silicosis and tuberculosis, every silicotic nodule is a menace, and every bit of finely powdered silica added to a tuberculous focus increases the tuberculous hazard. Some patients with slight involvement seem to carry considerable silicosis or modified silicosis without disability or impairment of health. There is evidence of individual variation among patients. There is also a difference of time at which disease appears, depending on the amount of silica, the amount of coal dust, the age of the patient and the state in which the silica exists. The diagnosis of the second stage of silicosis by means of the x-rays is relatively easy. In conditions that have not yet attained the second stage and any of the forms of modified silicosis (especially tuberculosilicosis) a diagnosis by means of the x-rays is as likely to be wrong as right. The clinical findings alone are of little use except to rule out other diseases. The history of exposure and the pathologic and chemical studies are the only certain means of diagnosis.

DISCUSSION

V. LEVINE. The micro-incineration method has been used to advantage to detect silicon in lymph nodes. I have in mind a patient who died of ulcerative tuberculosis. This patient had one large lymph node in the upper lobe of one lung, and by means of the micro-incineration method silicon was readily detected, whereas there was no silicosis in the remaining portion of the lung.

P. R. CANNON. Was there evidence of silicosis in the spleen, liver and bone marrow?

S. R. ROSENTHAL. Are the hilar lymph node tissues entirely destroyed in silicosis?

H. C. SWEANY. We now use a petrographic microscope which is valuable in differentiating silicon from other closely related doubly refractive substances. No silicosis was found in the livers or spleens of our patients with silicosis of the lungs. In many lymph nodes the lymphoid tissues are entirely destroyed, whorls of fibrous tissue are produced, and the lymph channels are blocked. In anthracotic lymph nodes the lymphoid tissue is destroyed to some extent, but no whorls are produced, and there is no blockage of the lymph channels.

FLUCTUATIONS IN BASOPHILIC AGGREGATION COUNTS WITH METEOROLOGIC ALTERATIONS. G. HOWARD GOWEN

Daily basophilic aggregation counts were made on four normal young laboratory workers according to the McCord method during the entire month of October 1934. When the daily counts were expressed graphically there were daily fluctuations with definite peaks. In all four cases the peaks coincided rather accurately. When these graphs were compared with a meteorograph for October 1934 it was

found that the basophilic aggregation peaks occurred in most instances at those periods when the barometric pressure was high and the temperature low. Such weather conditions are termed "polar infalls," and it has been definitely shown that when they occur there is a blood pressor effect. These pressor episodes and their effects are reflected by the bone marrow in a moderate summation in the increased production of young cells. Inasmuch as the presence of basophilic substance has been interpreted as one of the most constant characteristics of immature red cells it is not unreasonable to assume that possibly the stimulation of bone marrow resulting from the pressor effects produced by the polar infalls results in an increased production and liberation of red cells exhibiting basophilic aggregations and in this way accounts for the daily fluctuations and peaks. In the preliminary basophilic aggregation counts made on persons entering an industry where there is a lead hazard the foregoing facts should be taken into consideration in evaluating the results of such counts.

BUFFALO PATHOLOGICAL SOCIETY

Joint Meeting with the Buffalo Academy of Medicine, April 24, 1935

KORNEL TERPLAN, *President, in the Chair*

W F JACOBS, *Secretary*

MASSIVE MUCOID CARCINOMA OF THE STOMACH W F JACOBS

A white man, 62 years of age, complained of pain on swallowing food, with relief on regurgitation, and of loss of weight and tarry stools. A bulging mass was palpated in the upper part of the abdomen extending inferiorly to 2 finger-breadths above the umbilicus and blending laterally with the spleen and liver. Roentgenograms revealed narrowing of the lower end of the esophagus, irregularity of the cardiac end of the stomach and limited mobility. In a biopsy specimen obtained by esophagoscopy the stratified epiderm was acanthotic with scattered polymorphonuclear leukocytes, plasma cells and lymphocytes. In the subepithelial tissue could be made out an infiltrating mucoid carcinoma with signet ring cells and an abundant mucinous stroma. The patient died suddenly.

A finely granular gelatinoid ooze escaped from the abdominal cavity. The omentum was distinctly thickened. It was free, however, and covered coils of small intestine. The serosa of the small intestine was smooth and glistening. In the ileocecal area were situated two masslike accumulations of mucoid material. The lower end of the esophagus was converted into a narrow rigid tube. This portion of the esophagus and the fundus of the stomach formed the large mass which was palpated clinically. The stomach up to the antrum was a thick-walled rigid tube. The wall measured from 4 to 5 inches (about 10 to 13 cm). The mucosa here was ulcerated. The serosa was covered with mucoid nodules.

The peritoneum covering the under surfaces of the diaphragm and the pancreas and spleen exhibited a mucoid tumor and was from $\frac{1}{4}$ to $\frac{1}{2}$ inch (0.64 to 1.27 cm) thick. No metastases were found in the lymph nodes or in the liver. Histologically the tumor was a mucoid carcinoma with signet ring cells and mucoid degeneration.

Death was due to pulmonary embolism.

The massive involvement of the stomach and the absence of metastases in the lymph nodes were considered noteworthy.

EFFECT OF INJECTION OF BILIRUBIN AND BILE SALT ON THE VAN DEN BERGH REACTION NORMAN HEILBRUN and ROGER S HUBBARD

Pure bilirubin in amounts varying from 30 to 100 mg was dissolved in tenth-molar sodium carbonate and injected intravenously. Direct van den Bergh reac-

tions were carried out on a specimen of plasma taken before the pigment was given (the control) and on specimens drawn five minutes after the injection. When small amounts of the material (from 30 to 50 mg) were injected into normal subjects the reaction was invariably delayed, but larger amounts (from 75 to 100 mg) produced an immediate reaction (color developing within from 10 to 30 seconds). When the pigment was injected into patients with severe hemolytic jaundice an immediate reaction was produced, although the reaction of the control was delayed.

In some instances a preparation of bile salt (20 cc of a 20 per cent solution of sodium dehydrocholate) was injected either by itself or with the bilirubin. In four of five experiments this lessened the time before the appearance of the van den Bergh reaction or, if the reaction was already immediate, it significantly increased the intensity of the color produced.

Book Reviews

The Patient and the Weather By William F Petersen, M D, with the assistance of Margaret E Milliken, S M **Volume II Autonomic Dysintegration** Price, \$5 Pp 530, with 249 illustrations **Volume III Mental and Nervous Diseases** Price, \$5 Pp 375, with 192 illustrations Ann Arbor, Mich Edwards Brothers, Inc, 1934

The publication of volumes II and III of Petersen's series of monographs on "The Patient and the Weather" has preceded that of volume I Although the later volumes contain many references to presumably fundamental material in the first volume, each starts with a restatement of the author's concept and may be read independently of any other volume

Forming the underlying groundwork of Petersen's concept is the view, now generally accepted, that the vascular system, especially its important peripheral bed, is in a constant state of change and flux This vascular play may involve entire organs or larger areas of the body It may and does constantly take place in the terrain of terminal arterioles, neighboring fields need not be in the same state at the same instant

Stricker has made this mechanism and especially its state of vascular constriction or spasm the basis of much that makes up the field of pathology For him the mechanism is a neurovasomotor one Petersen goes much further and would make the rhythm of the vascular bed the result of many factors, both endogenous and exogenous, which mediate their effects through general or local chemical or physicochemical changes In the biologic rhythm, the most obvious manifestation of which is vascular change, Petersen recognizes two antipodal states or phases, which, for the sake of brevity, he terms the ARS and COD phases The first is characterized by tissue anabolism and alkalinity, tissue reduction and vascular spasm These major alterations are associated with an increasing systolic pressure, increasing tissue acidity and capillary permeability, a decreasing carbon dioxide content, an increasing p_H and potassium-calcium ratio and an increasing cholesterol content The ARS phase goes over gradually or abruptly into the COD phase The tissues and vessels are stimulated by relative anoxemia The author looks on the ensuing vasodilatation as an active process resulting from relative anoxemia, most pathologists have perhaps been in the habit of thinking of vascular dilatation as a more passive process The COD phase is characterized by tissue catabolism and oxidation and vascular dilatation These are associated with decreasing acidity and capillary and membrane permeability, a decreasing diastolic blood pressure, vascular dilatation, an increasing carbon dioxide and cholesterol content and a decreasing p_H and potassium-calcium ratio These alterations are relative

This interplay of phases is a fundamental manifestation of the living organism It is a highly labile, autonomic and automatic mechanism that makes life possible The person who is termed normal is attuned and adjusted to his vascular and tissue rhythm In any local terrain excessive reactivity may exhibit results that an old-fashioned pathologist would be inclined to include under such heads as tissue stimulation, hyperplasia, degeneration, atrophy, fibrosis, necrosis and inflammation The focal reaction may be a factor in bacterial localization and in the resulting chain of events that characterize infection If the person is actually or potentially inadequate, the widest variety of abnormal physical or psychic states may result All disease would appear to be traceable to abnormality of the biologic rhythm

Although the rhythm is influenced by a wide variety of factors both internal and environmental, the most important of these would appear to be the weather Weather is not merely a matter of heat and humidity, which are the frequent subject of gibe and jest It includes barometric pressure, velocity and direction of air currents, precipitation and electronic atmospheric ionization, as well as temperature and humidity Of greatest importance are the brusque cyclonic changes that

reveal themselves in a change from a warm to a cold front or vice versa "The human organism changes from day to day and this change is related to the meteorological environment It [the human biologic mechanism] is a mechanism that must constantly be adjusted to myriad environmental influences, the chief ones being meteorological" Not only does the weather affect the individual from the time that he begins his more or less independent existence on this troubled earth following the severance of his umbilical cord, but it hounds him in his mother's womb and may cause malformation It has been said that the best assurance for success in life is the proper selection of one's prospective parents But one should do more than that One should admonish one's future parents that they should exercise judgment in the performance of their marital rites and select a proper time for one's conception Particularly if one is going to be obsessed by the ambition to be president of these United States he must urge his parents to be careful during August and September, February is the ideal month for breeding presidents

Volume II "deals with the effects of meteorological alterations on the unstable individual It includes a study of migraine, epilepsy, eclampsia, miscarriage, colitis, the sensitizations, the more or less fundamental problems of the mechanisms involved, the focal reaction, the importance of terminal vascularization, the rôle of anoxemia, etc" The volume begins with five opening chapters that have the following headings "The Organism as a Cosmic Resonator", "Vascularization", "The Focal Reaction and Biological Rhythm", "Penetration of Bacteria and the Localization of Disease", "Focal Infection" Then follows a discussion of headache and migraine, with eleven detailed case studies This is followed by chapters on epilepsy, eclampsia and premature delivery, mucous colitis and gastric ulcer, the neuroses, urticaria, asthma, arthritis, glaucoma, the ear, the teeth, alopecia areata and therapeutic implications

Volume III "presents studies that have to do with mental and nervous disease" The eight chapters of the introductory first part have the following titles "General Discussion", "The Problem", "The Focal Reaction in the Psychoses", "Oxidation", "Malformations", "Mental Superiority—Insanity—Inferiority", "Seasonal Conception", "Suicide" Part II is devoted to a discussion and detailed case studies of certain psychoses, especially schizophrenia and the manic-depressive psychosis Part III takes up multiple sclerosis, tabes and dementia paralytica, poliomyelitis and meningitis as nervous diseases in which the localization of the disease process is influenced by the focal reaction

Each volume is illustrated by numerous graphic charts and maps For each case presented in detail there is, in addition to numerous smaller graphs, a "comprehensive graph," the comprehensiveness of which interferes somewhat with its comprehension Each graph has a meteorograph with six meteorologic factors Beneath this the observations made on the subject are graphically presented These observations, which may cover twenty or more different items, include, to select a graph at random, the reaction to the intracutaneous injection of epinephrine hydrochloride, the reaction to the intracutaneous injection of histamine, the dermographic reaction time, the McClure-Aldrich disappearance time, the systolic blood pressure, the diastolic blood pressure, the leukocyte count, the basal metabolic rate, the blood chlorides, the blood cholesterol, the blood sugar, the phosphates, the carbon dioxide content, the pH , the calcium-potassium ratio, the potassium, the calcium, the serum protein and the blister time The character of the observations made varies with the individual case The use of the same unit to represent 1 mg of one chemical substance and 10 mg of another results in disproportionate magnification of the variations of those constituents that have a small amplitude of variation

Petersen presents his material in a fascinating style that makes it difficult to lay aside a volume to gain the necessary sleep for the next day's work To say that writing is fascinating is to bestow the highest praise But the gentle art of book-reviewing demands the discovery of a fly in the ointment So the reviewer turns entomologist, and perhaps even etymologist This reviewer does not like, in a printed discourse, the abbreviation "T B" for the disease tuberculosis, nor

does he think that it is conducive to international amity to refer to Japanese as "Japs" He can find no lexicographic warrant for "haemopoetic," although poesis and poesy ultimately go back to the same Greek word meaning to make, or for "mucous" used as a noun, or for "neurophagia," "amytropic" and "hypospades," or "habitation" for habituation "Albuminurea" is evidently a misprint for albuminuria Schonlein, Strumpell, Losung, Fallung are without the umlaut, if this is because the typewriter used in the preparation of the master script did not have this character, the difficulty could have been met by the use of the proper diphthong The name of the reviewer's classmate is Loevenhart, not Loewenhardt Eyes are described as "wide and starry", the poet may speak of starry eyes, but for the physician eyes are staring Some errors are grammatical in character, such as the use of a singular verb with a double subject and the use of "lead" as the past tense of to lead "The fundi showed a salt and pepper fundus" may cause almost as much reaction in the leptosome reader as a polar infall Petersen affects the prefix "dys" for dis If the gastro-enterologist may write dyspepsia and the gynecologist dysmenorrhea, the author may be permitted to have a preference for "dysintegration," although this spelling does not have the sanction of usage

It is not to be supposed that the examples just given have been sought out with malice aforethought and a fine-tooth comb They have been encountered in the hurried survey that a reviewer makes of a book There must be many more such flaws in what is otherwise delightful reading The reviewer fears that they are evidence of carelessness

Petersen's presentation of his wealth of material is a convincing statement of his thesis After a volume has been laid aside and one has indulged in a little meditation and cogitation, one begins to wonder if an entire system of medicine or perhaps even of human biology is to be based on the weather Doll of Berlin has recently presented a study in which, although he admits a correlation between terrestrial meteorologic conditions and the state of the human body, he maintains that the latter is not dependent on the former, but that both the weather and the state of the body reflect electronic changes brought about on the earth by solar eruptions If the weather-vane and the barometer are to replace the plexor and the stethoscope, perhaps after we have learned more about the effects of other planets on our own the conical cap of the astrologer will replace the mortarboard of the academician

The author has presented the age-old conversational topic of the weather in a new and scientific manner The books themselves present the now old art of printing in a new format In the process, which is termed lithoprinting, a photographic electroplate of the typewritten page is made, and the inked impression is transferred to paper by means of a rubber roller The reviewer has found the type much easier to read than the usual print of books or newspapers The process lends itself well to the reproduction of illustrations and graphs

GROSS AND HISTOLOGIC CHANGES IN THE KNEE JOINT IN RHEUMATOID ARTHRITIS

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In previous papers¹ we presented the gross and histologic changes that are observed in joints with advancing age and in certain infectious conditions. It was pointed out that the changes which occur with advancing age are identical with those which have been described repeatedly as being characteristic for degenerative arthritis. It was shown that in the different infections the type and extent of the joint lesions depend on the mode of infection, that is to say, on whether it starts as a metastatic synovitis or appears in the joint by extension from the bone marrow or from the exterior. Degenerative arthritis begins in the articular cartilage and involves the bone and occasionally the synovial membrane secondarily. On the contrary in the infectious group the lesions begin most often in the synovial membrane, less frequently in the bone. In view of these findings the changes found in three cases of rheumatoid arthritis were studied to compare the lesions in this condition with those of degenerative and infectious arthritis. We obtained autopsies in two of the cases, in one the process was in a comparatively early stage, in the other far advanced. The early case occurred in a patient who died from cinchophen poisoning, and for the material we are indebted to Dr. Timothy Leary. The material from the third case was obtained through the kindness of Dr. Tom K. Richards, who resected the knee joint of the patient.

REPORT OF CASES

CASE 1—A white woman, aged 40, complained of arthritis of both knee joints and of the interphalangeal joints of both hands. This condition had bothered her at varying intervals for a period of two years. During the week before admission she took 30 capsules of cinchophen hydro-iodide and then hemorrhages appeared beneath the superficial layer of the skin of the face, about the ankles, wrists, legs,

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1 (a) Keefer, C. S., Parker, F., Jr., Myers, W. K., and Irwin, R. L. *Arch. Int. Med.* **53**: 325, 1934, (b) *Arch. Path.* **17**: 516, 1934. (c) Keefer, C. S., Parker, F., Jr., and Myers, W. K. *ibid.* **18**: 199, 1934.

arms and trunk. For two days before entry into the hospital there had been symptoms of a pulmonary infection with fever, cough and expectoration.

On examination the temperature was 103 F, the pulse rate 120 per minute and the respirations 45 per minute. There were extensive purpuric lesions over the face, arms, legs and trunk. There was no jaundice. The lungs showed evidence of bilateral bronchopneumonia, and the interphalangeal joints showed fusiform swelling and enlargement with some pain and tenderness on pressure and limitation of motion. The knees showed periarticular swelling and thickening without any excess accumulation of fluid. They were painful on motion and pressure. The red blood cell count was 4,447,000 per cubic millimeter, the hemoglobin, 50 per cent, the white blood cell count, 6,900. The urine showed slight amounts of albumin, an occasional leukocyte and a few red blood cells and granular casts. The bleeding time was 55 minutes (Duke) and the clotting time 25 minutes (capillary tube method), which is normal.

The clinical course was one of progressive failure with signs of bronchopneumonia, anemia and leukopenia with agranulocytosis. Death occurred three days after admission. The clinical diagnoses were drug intoxication, bronchopneumonia, rheumatoid arthritis, agranulocytosis and secondary anemia.

The anatomic diagnoses were dermatitis medicamentosa, purpura, organizing pneumonia and abscesses of the lung. The liver was normal on both gross and microscopic examinations.

Gross Examination of the Knee Joint—The synovial membrane and articular surfaces of the right knee joint were removed for study. The examination of the gross specimen revealed the following features. The synovial membrane and the underlying connective tissue were increased in thickness. The surface was irregular, and the membrane projected into the joint cavity as long tags of tissue. Wherever there were depressions or erosions in the articular cartilage the synovial membrane had extended into them and attempted to fill the cavities. In several areas, especially over the posterior aspect of the lateral condyle of the femur the synovial membrane had grown over the surface and had become adherent to it. The semilunar cartilages were difficult to recognize as separate and distinct structures owing to the great amount of synovial membrane that had overgrown and surrounded the cartilage.

The articulating surface of the patella showed fibrillation and erosion of the cartilage on the medial horizontal facets. About the edge the synovial membrane appeared in long thin tags, and over the median surface it was adherent to the cartilage. In the patellar groove of the femur the cartilage was irregular and fibrillated, and at the periphery the synovial membrane had grown over the cartilage. On the lateral condyle there was a small hole in the cartilage which communicated with a small cavity extending into the bone. Over a large area of the articulating surface of the medial femoral condyle there was a complete absence of cartilage, and the underlying bone contained several small holes communicating with the surface. The posterior part of the lateral condyle of the femur was covered with a thick layer of synovial membrane and was everywhere irregular. There were some areas of erosion of the cartilage and a small necrotic area in the underlying bone.

The articulating surface of the lateral condyle of the tibia showed extensive erosion and fragmentation of the cartilage. The synovial membrane had grown over the area of the condyle that was covered by the semilunar cartilage. There were irregularities in the articular surface and fibrillation of the cartilage in the vertical plane. The medial condyle showed extensive destruction of the cartilage with marked overgrowth of synovial membrane and small holes in the underlying bone.

Histologic Examination—Microscopic examination showed the synovial membrane to be much thickened with an increased number of synovial cells (fig 1). There were numerous vascular papillary projections, and in places there were areas of perivascular infiltration with lymphocytes and numerous multinucleated cells. Some of these papillary projections were necrotic. In some places both the papillary projections and the synovial membrane proper showed loss of synovial cells with a deposition of fibrin, in others, a meshwork of fibrin occurred between the synovial cells. In one section there were papillary projections of fibrin which showed beginning organization at their bases. In the subsynovial tissue there was a marked infiltration with lymphocytes, rare giant cells of the foreign body



Fig 1—Section of synovial membrane ($\times 95$) showing proliferation of synovial cells and infiltration with lymphocytes and plasma cells

type and macrophages, some containing blood pigment. In the fat there was marked perivascular infiltration with lymphocytes. The striated muscle about the capsule showed degeneration and atrophy.

No bacteria were seen in the synovial membrane in sections stained by the Gram-Weigert method.

Sections from the cartilage and bone showed defects, fibrillation and thinning of the cartilage. The cartilage was necrotic in places, as was the underlying subchondral bone. In one area, the joint surface was composed of a layer of dense vascular connective tissue covered with synovial cells and infiltrated with lymphocytes and plasma cells—evidently an ingrowth of the synovial membrane from the periphery. Beneath this connective tissue the articular cartilage had for

the most part disappeared, leaving the subchondral bony plate impinging on the fibrous tissue layer. In a few places remnants of the cartilage could be made out, and these apparently were undergoing dedifferentiation into connective tissue. There were small cavities and multilocular cysts in the marrow. The cysts were filled with fatty macrophages and blue-staining granular material. The walls were made up of hyaline material and surrounded by connective tissue. In the region adjacent to the cysts there was a perivascular infiltration with lymphocytes, and the bone was in part necrotic. Osteoclasts were numerous in relation to both the degenerated and the living bone. The origin of this type of cyst was shown by some earlier lesions which consisted of necrosis of fat tissue, which was taken up by macrophages and then surrounded by connective tissue. The debris in some of the cysts consisted of small pieces of cartilage which were partly necrotic. They were surrounded by mononuclear and polymorphonuclear leukocytes. The connective tissue of the wall of the cavity was continuous with the connective tissue of the synovial tissue which covered the joint surface at this point.

In this case there were changes in the synovial membrane with signs of chronic inflammation, loss of cartilage with necrosis and cyst formation in the underlying bone. In the cartilage and bone there were many changes characteristic of degenerative arthritis. This was anticipated from our previous observations and the age of the patient. There were, however, changes in the synovial membrane and periarticular tissues which are not seen in degenerative arthritis but which are characteristic of rheumatoid arthritis.

CASE 2—A woman, aged 60, was admitted to the hospital on account of loss of memory and disturbed mental condition. An accurate history was not obtained but it was learned that she had been unable to get out of bed for five years preceding her death on account of severe chronic arthritis which had produced crippling.

On examination she was found to be underweight and emaciated. The mucous membranes were pale, and there were decubitus ulcers over the buttocks. There was a senile emphysema and rales at the bases of both lungs. The joints of the hands and feet, the ankles and the knees showed marked deformity and periarticular thickening. There was no anemia, but a moderate leukocytosis with the leukocyte count varying between 11,100 and 16,500 per cubic millimeter. On the second day after admission signs of bronchopneumonia appeared over the lower lobes of both lungs. Death followed ten days later.

The clinical diagnoses were chronic bronchitis, emphysema, bronchopneumonia and chronic rheumatoid arthritis. The anatomic diagnoses were bronchopneumonia, bilateral acute bronchitis, chronic arthritis (rheumatoid) of the wrists and knees, cardiac hypertrophy, old fibrous peritonitis and pleuritis.

Gross Examination—The knee joints were opened and the articular surfaces and synovial membrane removed for study. The following observations were made on the gross specimens.

On opening the knee joints the periarticular tissue and synovial membrane were found to be greatly thickened. There was a moderate amount of thick exudate containing old blood pigment. Since both knee joints presented practically the same picture they are described together. The articulating surfaces of the

patellae were almost completely obscured by a great thickening and overgrowth of synovial membrane that had invaded the joint cavity from the periphery and had grown over the surface. The entire surface of the femoral condyles was greatly distorted and irregular. Here and there the cartilage and bone projected from the surface, leaving areas of elevation and depression. At no point was the surface smooth. The surface resembled a relief map of a very rugged country with many hills and valleys. In some areas there was great atrophy of the cartilage, and the superficial layers resembled fibrous tissue. At the edges the synovial tissue had invaded the joint cavity and covered the surface of the cartilage.

The articulating surfaces of the tibiae were reduced in size and distorted. This was due to the great overgrowth of synovial membrane and fibrous tissue over the semilunar cartilages and the articulating surfaces. Indeed, the semilunar cartilages could not be recognized as distinct structures. The articular cartilage was thinned and irregular and replaced by fibrous tissue.

Histologic Examination—The extent of the changes in the synovial membrane varied considerably from one portion to another. In the regions showing the least involvement the surface of the membrane was covered with delicate papillary projections made up of vascular connective tissue covered with flattened cells and containing occasionally a few lymphocytes. The deeper layers of the synovial sac showed a slight perivascular infiltration with lymphocytes and plasma cells. Where the process was more marked the synovial surface was thrown into folds and was covered with coarse villous projections. These had a central core of fibrous tissue, often dense and hyaline, and contained numerous blood vessels, often accompanied by a perivascular infiltration with many lymphocytes and plasma cells. The synovial cells covering these projections were large, their processes prominent, and they tended to have a palisade arrangement, lying with their long axes at right angles to the joint surface. In places these cells were covered with a delicate layer of fibrin, in other areas they had disappeared and there was present a dense layer of fibrin, often hyaline in character. The connective tissue wall of the synovial membrane was thickened and was infiltrated with numerous lymphocytes and plasma cells, most markedly in the vicinity of the blood vessels. Scattered here and there were histiocytes containing hemosiderin.

The articular surface was covered in great part by rather dense vascular connective tissue which presumably had grown in from the synovial membrane at the periphery, as no fibrosis of the marrow was present. Scattered along the surface also were projecting nodules of cartilage and these, as a rule, were not covered by the connective tissue. These cartilaginous nodules showed at their bases islands of vascular connective tissue with ossification proceeding at the peripheries of these islands. Only a few areas of articular cartilage of the usual type were present, and these showed varying degrees of fibrillation and, as a rule, islands of ossification at their bases. Beneath the connective tissue covering the articular surface there was a thin layer of bone, often containing islands of cartilage which represented remnants of the deep layer of the original articular cartilage. The line of the articular surface was markedly distorted, showing areas of flattening and also projecting areas due to the cartilaginous nodules mentioned.

The bony layer described as underlying the connective tissue in part represented the original subchondral bony plate. However, much of this bony layer was newly formed, since the original subchondral layer could be made out some 3 or 4 mm beneath the surface. This construction of a new articular layer of bone has been described also in case 3. The process showed various stages

Where it was complete, the original subchondral bone existed merely as a thin layer with an occasional island of cartilage embedded in it. Where it was somewhat more recent, the bone surrounded islands of cartilage and these were penetrated by vascular, ossifying tissue. In one area the articular cartilage, which still formed the joint surface, was being invaded at both sides by such ossifying tissue so that there was a definite narrowing at these regions, suggesting that eventually this mass of cartilage would be separated into two pieces with the portion toward the articular surface finally forming the new bone layer in that region, and the deeper portion representing the line of the original subchondral bony plate as described. In a region adjacent to this area, this separation had already taken place, the intervening spaces between the two portions being filled with trabeculae of bone and fatty marrow. The process of the formation of the new articular surface has been described in detail in case 3 and it does not seem necessary to repeat it here, the changes were identical. The bone of the epiphysis in general showed atrophy, the trabeculae being much thinner than normal and more widely separated. The marrow was fatty throughout and showed no pathologic changes.

The alterations in the joints of this patient were much more marked than were those in case 1. The duration of the disease and the disability caused by it were greater. There was extensive destruction of the cartilage in addition to the reaction in the synovial membrane. The atrophy of the bone was extreme.

CASE 3—Gross Examination—The specimen consisted of the articulating surface of the tibia and the posterior aspect of the condyles of the femur, which were resected.

About the borders of the specimens the synovial membrane was greatly thickened and showed proliferation of the synovial membrane and the underlying connective tissue. In some areas the synovial membrane had grown over the cartilage to which it was firmly attached. The cartilage over the area of the tibial condyle subjected to the greatest pressure showed thinning and erosion. Over other areas the cartilage was discolored, was definitely wrinkled and projected in an irregular manner. It was soft and had lost its normal elasticity. Some parts of the surface were distinctly nodular (fig 3). These nodules were firm, were covered by a thin layer of cartilage and varied from 2 to 4 mm in diameter.

Histologic Examination—The surface of the synovial membrane was irregular and showed a considerable number of papillary projections made up of vascular connective tissue densely infiltrated with lymphocytes and plasma cells and covered with a layer of synovial mesothelium. The synovial surface elsewhere was in part intact and in part covered with a dense layer of fibrin with loss of the synovial cells. The intact synovial layer was made up in places of several layers of synovial cells, the processes of which were unusually prominent. In foci there was a thin layer of fibrin over the intact synovial membrane. Beneath the synovial cells, in the connective tissue, were numerous scattered lymphocytes, plasma cells, a few polymorphonuclears and multinucleated cells. The multinucleated cells had nuclei of equal size, often with a peripheral arrangement. The cytoplasm, which was acidophilic, contained numerous centrioles. These cells often showed several processes of considerable length occasionally such a process extended up between the synovial cells to the surface. No foreign bodies could be detected.

in their cytoplasm. Their exact nature was not clear. However, the type of nucleus and processes so closely resembled those of synovial mesothelium, and their close connection with the latter suggested that at least some of them, if not all, were of synovial origin. In the connective tissue beneath the region just described there were numerous large focal collections of lymphocytes and plasma cells and, in addition, in places there were macrophages containing hemosiderin. An occasional definite lymph follicle with an active germinal center could be found. The connective tissue of the capsule showed an occasional perivascular collection of lymphocytes. The capillaries beneath the synovial membrane were prominent, as were their lining endothelial cells. Elsewhere the blood vessels were not abnormal.

Only a small portion of the joint surface was covered with cartilage. This cartilage was thinner than normal; its surface was roughened with papillary projections which were often covered with fibrinoid material, and it tended to stain acidophilically. At its junction with the subchondral bony plate were several foci of vascular connective tissue which penetrated somewhat into the cartilage. The greater part of the articular cartilage had disappeared, and the joint surface was covered with a layer of rather dense connective tissue containing medium-sized blood vessels and infiltrated with numerous lymphocytes and plasma cells. This connective tissue layer was covered with synovial cells and was obviously an ingrowth from the synovial membrane. Where this connective tissue covered the articular surface no articular cartilage was present save occasionally a thin layer connected with the subchondral bony plate. Chondroid tissue and young cartilage could be found in connection with the subchondral plate and, in one area, embedded in the connective tissue layer.

As mentioned in the foregoing paragraph, the connective tissue layer was a continuation of the synovial membrane and was similar in structure. In places there were deposits of fibrinoid material on its surface and here the synovial cells had disappeared. Its surface showed numerous coarse papillary projections extending for a considerable distance above the joint surface. In several places it had grown down through defects in the underlying bone into the marrow for a short distance.

The subchondral bony plate showed a variety of changes. In rare places it persisted with its normal cartilaginous component but was covered with the connective tissue layer. In other areas the plate and cartilage could be found but the cartilage was covered on the joint side by a closely applied layer of bone containing a few islands of connective tissue. Elsewhere the plate and cartilage could be distinguished, as before, with a bony covering on the joint side, but here there were bony projections also at right angles to the bony plate, extending toward the joint and terminating in another plate of bone parallel to the original subchondral plate. This new bone contained fatty marrow and also islands of connective tissue. The most exaggerated example of this picture was seen in one section in which the old subchondral plate could be distinguished in its entirety, but intervening between this and the joint cavity were bony trabeculae and fatty marrow for a distance of 2 mm, with a new plate of bone underlying the connective tissue lining the joint. In other areas the subchondral bony plate had disappeared entirely, leaving bony trabeculae running approximately at right angles to the joint surface, which was covered with connective tissue. In a few places where small breaks in the plate were present the fibrous tissue from the articular surface had penetrated down into the marrow. Nodules of chondroid tissue were found projecting from the surface of the bone in the region of the subchondral plate. In one focus where the articular cartilage had been lost, leaving the underlying bone exposed, there were marked thickening of the bone

extending down for quite a distance and fibrosis of the marrow. As a result of these various changes the joint line was markedly irregular. The bony trabeculae of the epiphysis seemed thinner and more widely separated than normal.

There were a few scattered areas of normal hematopoietic tissue in the marrow. As described in the foregoing paragraph, invasion of the marrow by fibrous tissue from the articular surface had occurred in several areas. Also fibrosis of the marrow had taken place where there was marked thickening of the bone beneath regions where the articular surface was denuded. In connection with the latter, large focal collections of lymphocytes were found in the marrow spaces.

In this case as in case 2, extensive alterations were found in the synovial membrane as well as in the articular cartilage.

COMMENT

From these three cases it is evident that the constant lesion is in the synovial membrane and periarticular tissues. The other changes are secondary. Following the synovitis a series of changes take place that require study and analysis. They will be presented but before that is done it will be well to summarize the knowledge of the synovial lesions which are characteristic of the disease.

The first change observed in the synovial membrane is an increase in the number of synovial cells with marked thickening of the membrane. Here and there the synovial cells are destroyed and become covered with fibrin. In the synovial tissue there are collections of lymphocytes and plasma cells, macrophages and rare giant cells. These various cells collect in dense foci arranged about blood vessels in some areas and not in others (fig. 2). In the fat of the periarticular tissues there is an infiltration of lymphocytes about the blood vessels. The striated muscle about the capsule of the joint shows degeneration and atrophy. Following the proliferation of the synovial membrane and its overgrowth onto the surfaces of the joint, the articular cartilage frequently disappears. This has been ascribed by Nichols and Richardson² and Allison and Ghormley³ to a dual process. They maintain that as the synovial membrane passes over the articular cartilage it becomes adherent to it and finally destroys it. At the same time they describe proliferation of connective tissue in the subchondral spaces with an invasion and destruction of the cartilage.

From our observations on the disappearance of the articular cartilage we have come to the conclusion that it takes place as a result of a solution of the cartilage under the connective tissue that has grown onto the articular cartilage and by a dedifferentiation of cartilage into connective

2 Nichols, E. H., and Richardson, F. L. *J. M. Research* **21** 149, 1909.

3 Allison, N. A., and Ghormley, R. K. *Diagnosis in Joint Disease*, New York: William Wood & Company, 1931.

tissue We have not been able to convince ourselves that proliferation of connective tissue in the marrow of the epiphysis is a necessary and essential part of the pathologic process

The foregoing statements are based on the following observations When the cartilage under the connective tissue preserves the original characteristics of normal articular cartilage it appears to undergo solution As a general rule, however, the cartilage beneath the connective tissue is quite different in appearance from normal articular cartilage

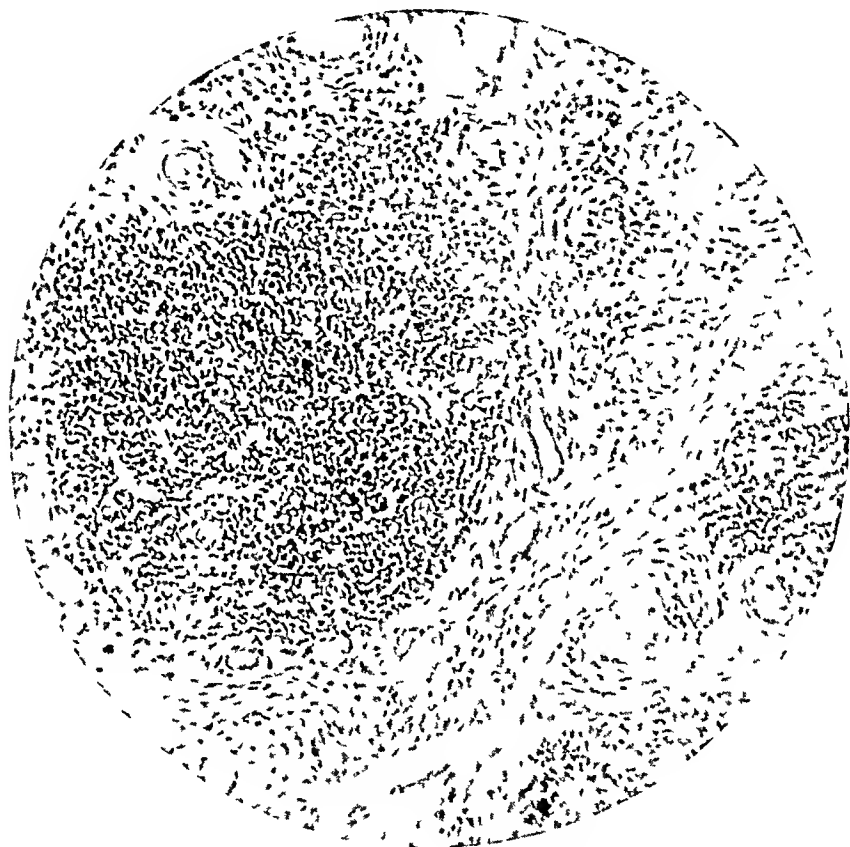


Fig 2—Section from subsynovial tissue showing infiltration with lymphocytes in foci about blood vessels and elsewhere ($\times 125$)

The intercellular substance stains with acid dyes, the cartilage cells no longer occur in groups but tend to become more separated and to have a clear zone about them All of these changes are more marked the nearer the overlying connective tissue is approached The other process that takes place is a conversion of the cartilage into connective tissue This was exceedingly common in the cases herein described In numerous sections it was found that cartilage was being converted into connective tissue, since young cartilage cells could be traced into the connective tissue where they became still more widely separated and more irregular in shape

Since the cartilage seemed to change into connective tissue so frequently the question arose whether this ever occurred without evidence of connective tissue growing over the surface of the articular cartilage. We have many sections bearing on this question, and it can be stated that it was not observed. There were some sections in which this apparently was not true, and they require comment. In a few sections, nodules of cartilage were seen projecting upward from the surface of the joint and they were uncovered by connective tissue, although connective tissue could be seen surrounding and between them (fig 3). The explanation of these nodules was that they represented a formation of



Fig 3—Section showing small nodules of cartilage projecting upward from the surface of the joint. They have been formed from the connective tissue which at one time covered the whole surface of the joint ($\times 4$)

cartilage from connective tissue which at one time covered the whole surface area. That this can occur there can be no doubt since in figure 4 it is seen that cartilage, bone and a new articular surface have been formed on top of old articular cartilage and this, in turn, is covered by connective tissue. In other words, when connective tissue grows over articular cartilage the latter either undergoes solution or becomes changed into connective tissue. The connective tissue which grows over the surface of the joint is also capable of forming new cartilage and finally bone.

In sections which failed to show any connective tissue growing over the surface of the joint there was no evidence of cartilage changing into

connective tissue and no marked proliferation of the connective tissue of the marrow. This was demonstrated clearly in a cross-section of the tibia. At the periphery of the joint surface the articular cartilage was covered by connective tissue and beneath it the cartilage was undergoing dissolution. The part of the cartilage which showed no connective tissue over its surface was normal in appearance except for the presence of fibrillation, an associated lesion due to degenerative changes.

The articular cartilage was destroyed in still another way. We have described the irregularity of the surface of the joint in these cases and have shown that this irregularity is due to a replacement of the articular cartilage by new cancellous bone, so that the surface consists of a very thin layer of bone, connective tissue or cartilage (fig 5). The remains



Fig 4—Section showing the original articular cartilage covered by connective tissue, new cartilage and bone. There is also evidence of destruction of part of the original articular cartilage with new bone formation ($\times 3$).

of the old subchondral joint line are seen several millimeters below the surface. The formation of this double line with cancellous bone intervening has been explained by some to be due to a proliferation of the perichondrium of the articular surfaces. The existence of perichondrium in adult cartilage can be doubted since there is very little evidence to show that it exists and we have never been able to detect tissue that could be differentiated as perichondrium in the joints which we have examined. We believe, however, that the appearance of a double line and the destruction of the cartilage can be explained on another basis, namely, that it results from a replacement of the articular cartilage by cancellous bone, and it progresses not from the subchondral plate by ingrowth of connective tissue into the cartilage but begins at the periph-

ery of the joint and spreads through the central portion of the articulating cartilage. In some sections one can trace the process from both ends. This is illustrated clearly in figure 6. As it progresses the subchondral bony layer becomes separated from the superficial layers of cartilage, and gradually the whole articular cartilage may become ossified and only remnants of the old subchondral line will be seen. That this process does not progress from below upward was confirmed by other sections in which the cartilage was being destroyed from the side and from the articular surface downward by ossification.

It seems clear, then, that the double line of cancellous bone results from the ossification of the articular cartilage, and that this is accom-

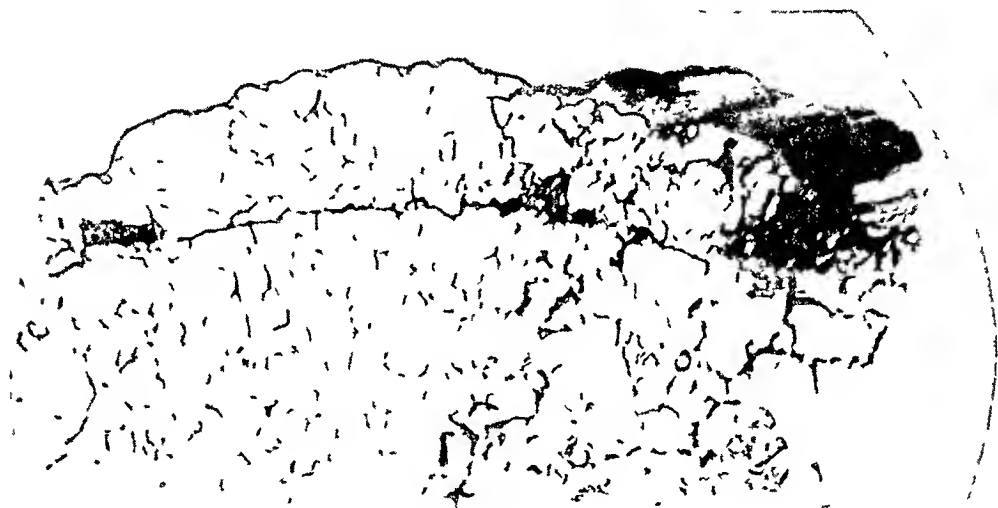


Fig 5—Section of the femoral surface of the knee joint showing complete ossification of the articular cartilage and the remains of the original subchondral line ($\times 4$). This is the end-stage of the process shown in figure 6.

plished by an ingrowth of ossifying tissue from the periphery of the joints.

There remains for discussion the question of the destruction of the cartilage by ingrowth of connective tissue from the epiphyseal marrow. In our previous paper¹² on the histologic changes in the joints with advancing age we called attention to the occurrence of fibrosis of the marrow especially when the cartilage on the surface had been destroyed. This increase in connective tissue of the marrow extended up to and over the affected surface and we interpreted this reaction as an attempt to repair the defect.

It was shown further that when the subchondral zone showed increased vascularization, a process which is common and which is pathognomonic of degenerative arthritis occasional islands of vascular connective tissue extended upward into the overlying cartilage, and new bone formation took place about them. It is necessary then, to take these findings into consideration when interpreting the changes that are seen in rheumatoid arthritis.

In many sections from these cases of rheumatoid arthritis it was demonstrated that the connective tissue that had grown over the surface of the joint frequently extended downward into the marrow after the cartilage and bone had been destroyed. This is seen in fig 7. In these

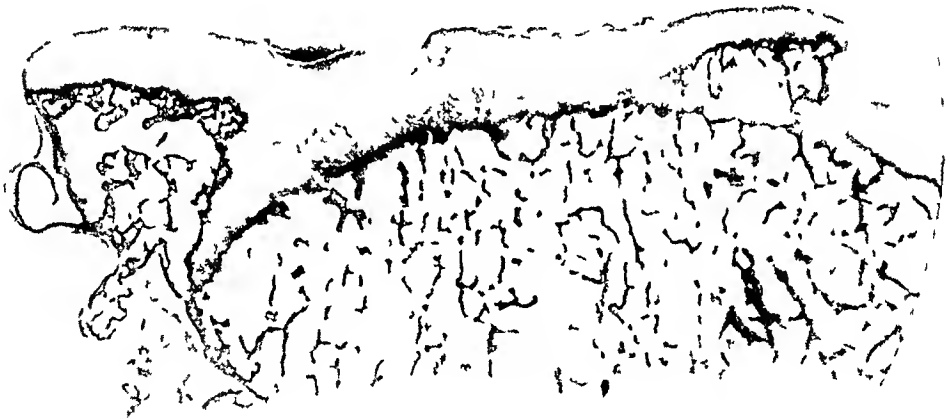


Fig 6—Section of the femoral surface of the knee joint showing ossification of the articular cartilage by a process progressing from the periphery of the joint ($\times 3$)

cases the connective tissue on the surface was always older in appearance than that seen in the marrow, and it could be traced from the surface into the marrow. The evidence was not convincing that any great amount of destruction took place by invasion of the subchondral plate by connective tissue from the marrow, so that we did not feel that this was of fundamental importance in the pathologic process.

In summary, then, it can be reasonably maintained that destruction of the articulating cartilage takes place by solution and by dedifferentiation of cartilage into connective tissue once its surface has been covered with connective tissue. Secondly, it is replaced with cancellous bone by a process of ossification from the periphery. The destruction of carti-

lage by connective tissue growing into the cartilage from the subchondral spaces is minimal and not a fundamental pathologic process in the disease. It is seen when new bone formation goes on in such areas.

Changes in the Bone—One of the outstanding features in these cases is the extreme degree of atrophy of the bone (fig 5). It is demonstrable by x-ray film during life, and when one examines the



Fig 7—Section of a joint showing disappearance of the articular cartilage following an overgrowth of connective tissue. In one area the connective tissue proliferation has extended into the marrow from above ($\times 36$).

bone histologically there is an amazing degree of such atrophy. The bone is reduced to a thin network resembling the finest lace. There is no evidence from the cases we have studied that the thinness of the bone is due to any process other than atrophy. That is to say, there is no indication of active bone destruction. We attribute this atrophy to disuse and not to a pathologic process causing dissolution of bone.

From the foregoing discussion there is justification for the belief that the fundamental process in rheumatoid arthritis is an inflammatory process in the periarthicular tissues and synovial membrane. The other anatomic changes are secondary to this essential change, that is to say, the destruction of cartilage, the atrophy of bone and new bone formation. A part of the process can be explained on the basis of disuse of a joint together with the loss of cartilage that occurs following the connective tissue overgrowth. We emphasize this point in order to call attention to the fact that any theory that attempts to explain the pathogenesis of rheumatoid arthritis must take into account the mechanism by which the changes in the periarthicular tissues come into being. The general pathologic changes are summarized in the table.

Pathologic Lesions in Rheumatoid Arthritis

Primary	Secondary	Miscellaneous
Synovitis	Destruction of cartilage	Lymphoid hyperplasia
Periarticular change with and without subcutaneous fibroid nodules	Atrophy of bone	Calcification of blood vessels
	New bone formation	Amyloidosis
	Subluxation	Disturbances of growth
	Ankylosis (fibrous bone)	Pigmentation of the skin
	Muscular atrophy	

The lesions in the cartilage which are so distinctive of degenerative arthritis are quite different from those resulting from destruction of the cartilage by overgrowth of connective tissue. We make this statement and emphasize it since it has been alleged with some confidence that both degenerative and rheumatoid arthritis⁴ result from the same underlying cause or group of causes. The origin of this view has as its basis the clinical observations that many patients with rheumatoid arthritis show degenerative changes in the cartilage. This is true but not surprising since a high percentage of persons after 40 years of age show degenerative changes in their joints. If rheumatoid arthritis develops after degenerative lesions are present, and this is not uncommon, then it naturally follows that the pathologic lesions of both conditions will be present together. Any one who has had the opportunity to study the gross and the histologic pictures of degenerative and rheumatoid arthritis cannot question that the underlying mechanisms in the two conditions are quite different. The picture of each is highly characteristic and can be recognized readily with a little experience. It is necessary to make the distinction since without it misunderstandings arise.

⁴ Archer, B. H. J. A. M. A. **102** 1449, 1934. See this paper for additional references to articles suggesting the unitarian theory.

SUMMARY

Gross and histologic examinations of the tissues from three cases of rheumatoid arthritis were made. The following points were brought out:

In rheumatoid arthritis the primary and essential lesion of the joint is an inflammatory reaction in the synovial membrane and periarticular tissues. The other changes are secondary to this process.

The articular cartilage disappears as a result of an overgrowth of connective tissue on its surface, dedifferentiation into connective tissue and ossification of the articular surface.

The atrophy of bone is not due to active bone destruction. It is dependent, we believe, on disuse and there is a loss of calcium in the bone which accounts for it. We suggest that this is the result of disuse.

The lesions of rheumatoid arthritis are easily distinguishable from those caused by degenerative changes and those resulting from infection of the joints with micro-organisms.

The theory that degenerative and rheumatoid arthritis result from the same underlying factors is untenable.

DIFFUSE CORTICAL CONTUSION OF THE OCCIPITAL LOBE

CYRIL B. COURVILLE, M.D.

LOS ANGELES

Contusion is one of the more common traumatic lesions of the brain. While there is some difference of opinion as to the exact mechanism of its production, it is quite generally agreed that in most instances it is a contrecoup and not a direct effect of the injury. Two notable exceptions are those cerebral contusions which occasionally result from depressed fractures of the cranial vault and cerebellar contusions secondary to linear fractures of the occipital bone which usually run near or into the foramen magnum.

There is a great variation in the size and character of cortical contusions. This variation is due to a number of factors: the size, shape and force of the traumatizing object, the mechanism and direction of its application to the head, and the peculiar anatomic characteristics of the region opposite to the point of impact. With few exceptions, contusions are the result of one type of mechanical force—the head in motion strikes a solid and relatively stationary object. They occur typically as the result of traffic accidents, either when one automobile collides with another or with some stationary object or when a pedestrian is struck by a moving vehicle.

The size of the contused area probably depends on the intensity of the applied force. The extremely severe contusion which results fatally within from twelve to twenty-four hours is often very extensive, affecting the opposite temporal and frontal lobes and extending deeply into the underlying white substance. On the other hand the contusion may be microscopic in size, evidenced only by tiny hemorrhagic effusions in the affected cortex.

The nature of the contusion depends largely on the anatomic relationship of the brain to the internal contour of the skull in the region. The shape of the contusion is also affected somewhat by the internal structure of the brain, particularly by the arrangement of the bundles of white fibers in the affected part. Three anatomic types of contusions are thus produced, the first with two subtypes: (1) the wedge-shaped temporo-frontal contusion with (a) subfrontal and (b) antero-lateral temporal subtypes, (2) the patchy and superficial dorsolateral contusion usually found in the opercular cortex and (3) the diffuse cortical contusion of the occipital lobe.

It is the purpose of this study to draw attention to the essential features of the third type of contusion which has not been described in the literature so far as I can discover. Certainly it must be familiar to coroner's pathologists in large metropolitan centers, but writers on the subject have not distinguished it from other types of cerebral contusions. The lesion appears as a diffuse reddish-brown discoloration of the involved convolutions. This coloration is due to the presence in the cortex of myriads of petechial hemorrhages. The lesion has been found invariably in the posterior portion of the cerebral hemisphere. The area involved varies considerably in size, from small patches of discoloration of the cortex, often at the depth of a sulcus, on the one hand, to a change in the cortex of the entire occipital lobe, on the other.

This study is based on observations in 8 such cases. The patients died after intervals of varying lengths in the Los Angeles County General Hospital, and the autopsies were performed by the coroner of the County of Los Angeles. The specimens were studied more in detail in the Cajal Laboratory. During the two and a half year interval during which the studies were made, a series of 241 specimens showing evidence of cerebral injury have been examined. Of this number 172 were found to show contusions of the brain. According to these figures, this type of lesion occurs in about 3 per cent of all cases of cranio-cerebral injury and constitutes only 4 per cent of cerebral contusions.

REPORT OF CASES

CASE 1—During an epileptic seizure a 57 year old man sustained an injury to the right side of the head in the frontal and parietal regions. He was admitted to the Los Angeles County Hospital on Jan 24, 1933. He was uncooperative and was discharged against advice on the following day. He complained of right-sided headaches for several days but did not seek medical attention. On February 2 he became mentally hazy, was unable to talk and soon lapsed into a comatose state. He was readmitted to the hospital on February 3.

He was found to be rather stuporous, reacting to painful stimuli with movements of the extremities of the right but not of the left side. The head was deviated to the left. The pupils were unequal, the left being larger than the right, both failed to react to light. There was marked spastic paralysis of the extremities of the left side. A hemorrhage in the interval being suspected from the history, a left subtemporal decompression was performed. A large amount of subdural blood clot was evacuated. He died with signs of bronchopneumonia on February 4, a few hours after the operation and ten days after his injury.

The autopsy was made by Dr. A. F. Wagner, who permitted a further study to be made of the cerebral lesions in the fixed specimen. A linear fracture was found in the right parietal region which extended into the squamous portion of the right temporal bone. The remains of an extensive subdural hemorrhage were found over the dorsolateral surface of the left cerebral hemisphere, the clot being thickest toward the base. The hemorrhage was found to have its source in an extensive laceration of the basilar surface of the left temporal lobe. There had

been a rupture of a large intracerebral hemorrhage through the cortex. A large cavity had been excavated in the central portion of the left temporal and occipital lobes.

The cortex of the entire occipital lobe had a peculiar plum color. The margins of the area were rather sharply defined. Over the crest of the convolutions the pia-arachnoid had been torn as though by a swelling of the cortex. The extent of this discoloration of the cortex is shown in the accompanying drawing (fig 1). On cut section the discoloration appeared at first to be limited to the cortex, but on closer study petechial hemorrhages were also observed in the subcortical white substance, which in some areas was softened and slightly discolored. In some regions the cortex seemed to be detached from the underlying white substance.

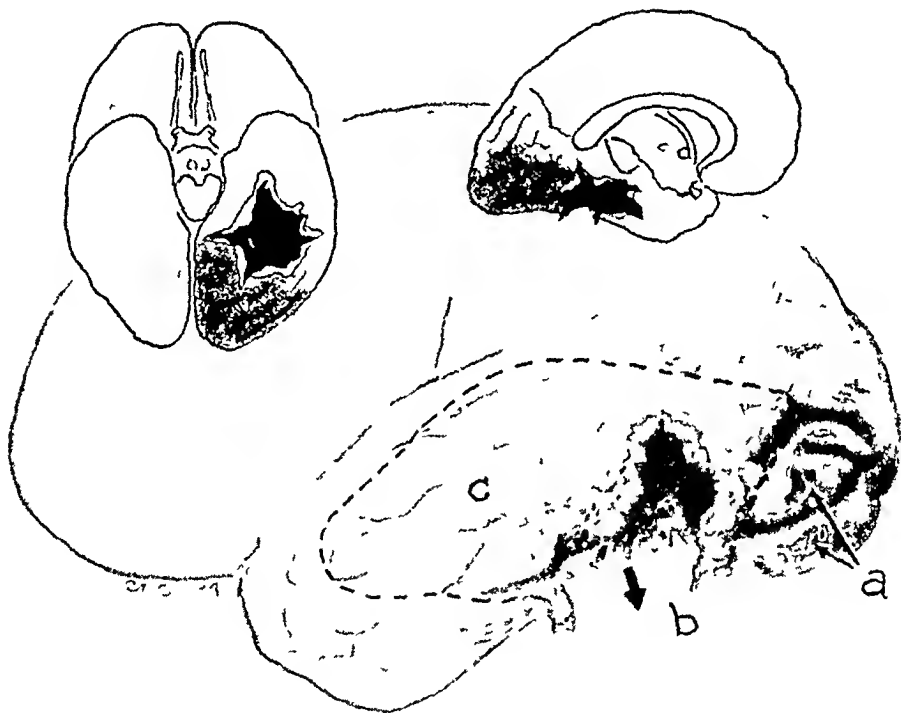


Fig 1 (case 1)—A drawing showing the location and extent of the diffuse contusion (a) ruptures of leptomeninges over convolutional ridges, (b) a laceration resulting from the rupture of an intracerebral hemorrhage through the cortex, (c) area within broken lines showing extent of intracerebral hemorrhage

Comment—There are several points of interest in this case which need to be stressed. While not germane to the problem at hand, the occurrence of an intracerebral hemorrhage so many days after injury and with the so-called “latent interval” calls attention to the possibility of such a lesion simulating an extradural or a subdural hemorrhage. This case is also of interest in that the cortex of practically the entire left occipital lobe was involved by the diffuse contusion, the most extensive lesion of the series.

CASE 2—A 46 year old white woman was rendered unconscious as the result of an automobile accident on June 5, 1934, and was admitted to the hospital in a state of shock several hours thereafter. When examined at this time she had partially regained consciousness. A laceration 10 cm long was found in the right parietal region, another 6 cm long was present over the vertex, and still another 4 cm long was located in the left temporal region. There were a few other superficial bruises of the skin of the extremities. No localizing signs suggestive of a focal cerebral lesion could be made out. She remained in a semistuporous state and died with symptoms of bronchopneumonia twelve days after her injury.

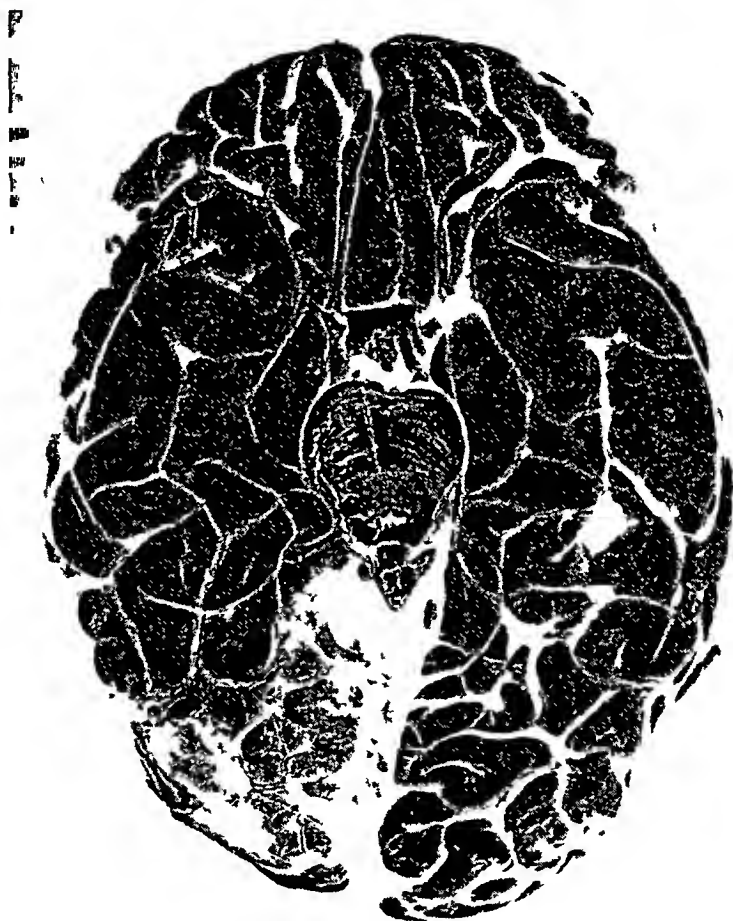


Fig 2 (case 2)—Extensive diffuse contusion of the basilar surface of the temporal and occipital lobes

At autopsy no fracture of the skull was found. There was a small amount of blood in the subarachnoid space in the right and left parietal regions, more on the left. There was a small contusion of the basilar surface of the left temporal lobe and others on the superior surface of the underlying cerebellum, that on the right being most extensive. A large portion of the basilar and medial cortex of the right occipital lobe presented the characteristic appearance of a diffuse cortical contusion (fig 2). The occipital pole and a small portion of the dorsolateral surface of this lobe were similarly affected. A few scattered petechial hemorrhages were also found in the centrum of the anterior portion of the left frontal lobe.

Comment—The extensiveness of the diffuse occipital contusion in this case is of special interest, particularly in view of the fact that the patient lived so long. Another interesting observation was the occurrence of the contusion of the superior surface of the underlying cerebellum. Such an occurrence is most unusual even in a large series of specimens presenting injuries.

In the following cases the cortical contusion was less extensive and only the essential points in the pathogenesis and pathology of the condition will be mentioned.

CASE 3—A 72 year old man died eleven days after being run down by an automobile. He was only slightly stuporous after his injury but became more so as time went on. A contusion of the right side of the head was found on examination. At autopsy there was observed a transverse linear fracture of the right temporal bone extending across into the middle fossa of the skull with local subdural hemorrhage. There was a severe contusion of the basilar surface of the left temporal lobe with local subdural hemorrhage. Petechial hemorrhages were present in the centrum of the right temporal and parietal lobes. Two small patches of diffuse contusion were noted (*a*) about the posterior end of the left superior temporal gyrus and (*b*) about a sulcus along the inferolateral margin of the occipital lobe.

CASE 4—A 63 year old man died twelve and one-half days after an assault by robbers. Following the injury there was bleeding from the right ear. After a short period of unconsciousness he recovered sufficiently to return to his work in spite of severe left-sided headaches. Ten days after the injury he became comatose and was admitted to the hospital for study. He died after a bilateral subtemporal decompression and partial evacuation of a left subdural hemorrhage. At autopsy no fracture of the skull was noted, but the remains of a subdural hemorrhage were found on the left side, together with a severe contusion of the medial part of the basilar surface of the left temporal lobe. Focal hemorrhages were also found in the right midbrain and in the pons. There was also a diffuse cortical contusion along the calcarine fissure on the left side.

CASE 5—A 60 year old man survived injuries resulting from an automobile accident for four and one-half days. He was unconscious for a short while, after which his mind was lucid for two days. During this time paralysis of the left arm was noted which was due to a fracture dislocation of the fourth and fifth cervical vertebrae. At autopsy, in addition to the fracture of the spine and injury of the cervical cord, a left subarachnoid hemorrhage and petechial hemorrhages in the left parieto-occipital centrum were found. A diffuse cortical contusion was also noted about the collateral fissure of the basilar surface of the left temporal and occipital lobes. There was no fracture of the skull.

CASE 6—A 41 year old man who fell from a moving automobile survived his injury for eight hours. After admission to the hospital on Dec 12, 1933, he was found to have spastic left hemiplegia. There was a laceration of the scalp in the occipital region. At autopsy a basal fracture of the skull and a severe contused laceration with hemorrhage into the left temporal lobe were found, associated with some local subdural hemorrhage. There was also a marked edema of the right temporal lobe resulting in herniation of the hippocampal gyrus through the

incisura of the tentorium with consequent compression and distortion of the mid-brain. The cortex lateral and caudal to the herniated hippocampal gyrus disclosed the typical changes of diffuse cortical contusion.

CASE 7—A 53 year old man sustained an injury to the head in an unknown fashion during a brawl on April 20, 1935. He was admitted to the hospital the following day because of persistent stupor. Examination disclosed a bruise over the vertex of the scalp, rigidity of the neck and equivocal plantar reflexes but no evidence of motor weakness or inequality of the deep reflexes. The patient remained in a semicomatose state and died eleven days after his injury. At autopsy a contusion of the scalp over the vertex was found but no fracture of the skull. A moderate left subdural hemorrhage, a minor right subarachnoid hemorrhage and severe contusions of the basilar surfaces of both frontal lobes and of the left temporal and parietal lobes were also found. A diffuse contusion had discolored the cortex on the medial and basilar aspects of the left occipital lobe as well as along the right calcarine fissure.

CASE 8—A 42 year old man was struck by an automobile while crossing the street on April 9, 1935, and was unconscious thereafter for a period of from fifteen to twenty minutes. He was admitted to the hospital the following day complaining of headaches. Evidences of bleeding from the right ear were found. He vomited repeatedly and was restless. Because of increasing drowsiness and dilatation of the right pupil a decompression of the right side was performed and a large subdural blood clot partially evacuated. Death ensued a few hours after operation and thirty-two hours after his injury.

At autopsy there was found an extensive linear fracture of the right side of the vault of the skull which extended into the base and a bruising of the right side of the scalp and the right temporal muscle. The remains of a very large right subdural hemorrhage were also found. There was a contusion of the tip of the right temporal and another of the dorsolateral surface of the left temporal lobe. A diffuse contusion of the cortex was found along the margin of a herniation of the right hippocampal gyrus into the incisura. This contusion also extended back along the calcarine fissure on this side. An old contusion of the cerebellar cortex was also noted, the result of a fracture of the occipital bone sustained five and a half years before.

PATHOGENESIS OF DIFFUSE OCCIPITAL CONTUSION

From a study of the clinical history and the observations at autopsy in this series of cases certain conclusions may be drawn as to the mechanical production of this type of lesion. Diffuse occipital contusion is found in persons over 40 years of age and usually after an interval of several days (exceptions cases 6 and 8). It occurs after blows to the head, after falls and after automobile accidents. It may occur without fracture of the skull, and when fracture is present there is no definite anatomic relationship between it and the contusion.

In order that the mechanism of the production of diffuse contusion may be understood two important observations should be mentioned. This type of contusion invariably occurs in the posterior portion of the cerebral hemispheres and is usually confined to the occipital lobe. The scalp wounds are likewise found over the posterior one-half of the head.

The first observation suggests that some regional anatomic peculiarity is responsible for this type of lesion. The absence of superficial lacerations of the cortex in the affected area such as occur when the bruise is due to contact with bone and the occurrence of such lesions along the free margin of the tentorium suggest, furthermore, the possibility that the arrangement of the dural reduplications may play an important rôle in the production of the diffuse contusion. This is also suggested by the fact that in most instances the contusion is in the cerebral cortex adjacent to one of these dural reduplications (exception case 1, in which contusion was associated with and evidently due to an extensive intracerebral hemorrhage).

In an attempt to evaluate the mechanical factors involved two possibilities must be considered. A primary type of lesion is apparently due to injuries sustained while the head is in motion, as is the case with other contusions. The contusion occurs predominantly, if not invariably, on the same side of the brain (cases 2, 3 and 5). When the vertex of the skull is injured primarily, both sides of the brain may be contused (case 7). A secondary type of contusion results from persistent pressure of the affected area against a dural reduplication, the pressure resulting from edema of one hemisphere (case 6), intracerebral hemorrhage (case 1) or subdural hemorrhage (cases 4 and 8). The lesion then occurs on the side affected by the cause of the pressure. The side so affected is not necessarily the side of the head originally injured.

The primary type of lesion is apparently produced by a sudden obstruction of the afferent veins of the cortex and subcortex or occlusion of these channels, the secondary type, by long-continued pressure against the relatively elastic dural reduplications. In the primary type the contusion is due to a sudden distortion of the occipital lobe on the side of the injury within a chamber the walls of which are formed in part by the smooth-surfaced dural reduplications (falx, tentorium). The occipital lobe is thrown against these reduplications and a contrecoup results. The relative elasticity and smoothness of these walls account no doubt for the absence of gross disorganization of the cerebral cortex common in other types of contusions. The sudden reversal of the current in the venous channels very likely accounts for the rupture of cortical and subcortical vessels and the formation of myriads of cortical and subcortical hemorrhages. Because of this interference with the circulation, softening of the cortex and underlying white substance ultimately results. The quantitative variation in the blood supply of the gray and white matter probably accounts for the difference in degree of softening and for the tendency for the cortex, after a short interval, to become separated from the underlying white substance.

PATHOLOGIC ANATOMY OF DIFFUSE CORTICAL CONTUSION

Diffuse contusion is found only in the occipital lobe or its immediate environs. It varies in extent from a small discolored area usually situated about the depth of the sulcus in one case to involvement of

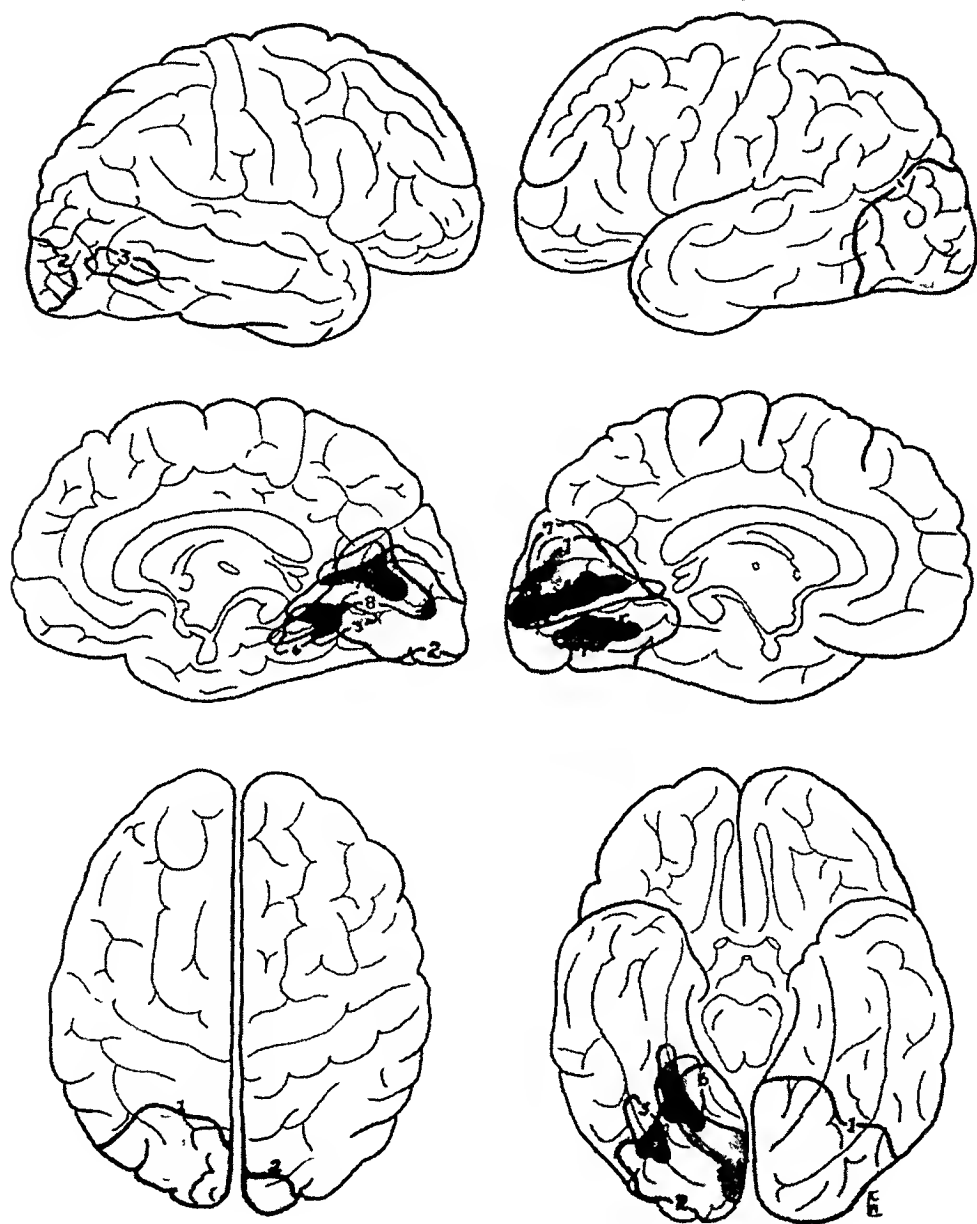


Fig 3—Drawings showing locations of diffuse contusions in cases 1 to 8. The numbers on the lines correspond to the case numbers.

practically the entire occipital lobe in another (fig 3). This is true of both the primary and the secondary types. The involved region is not uniformly affected; the discoloration being most marked where the greatest degree of hemorrhagic infiltration occurs.

Only rarely are the overlying leptomeninges torn and then usually on the dorsolateral surface where the inner surface of the skull does not present a uniformly flat surface (case 1). Viewed externally the affected cortex at first assumes a mottled red appearance, after fixation it assumes a characteristic plum color. It resembles quite closely red softening secondary to thrombosis of the superficial cortical veins. The lesion is essentially a hemorrhagic softening particularly of the cortex (fig. 4). The yellow softening of the subcortex is more likely due to

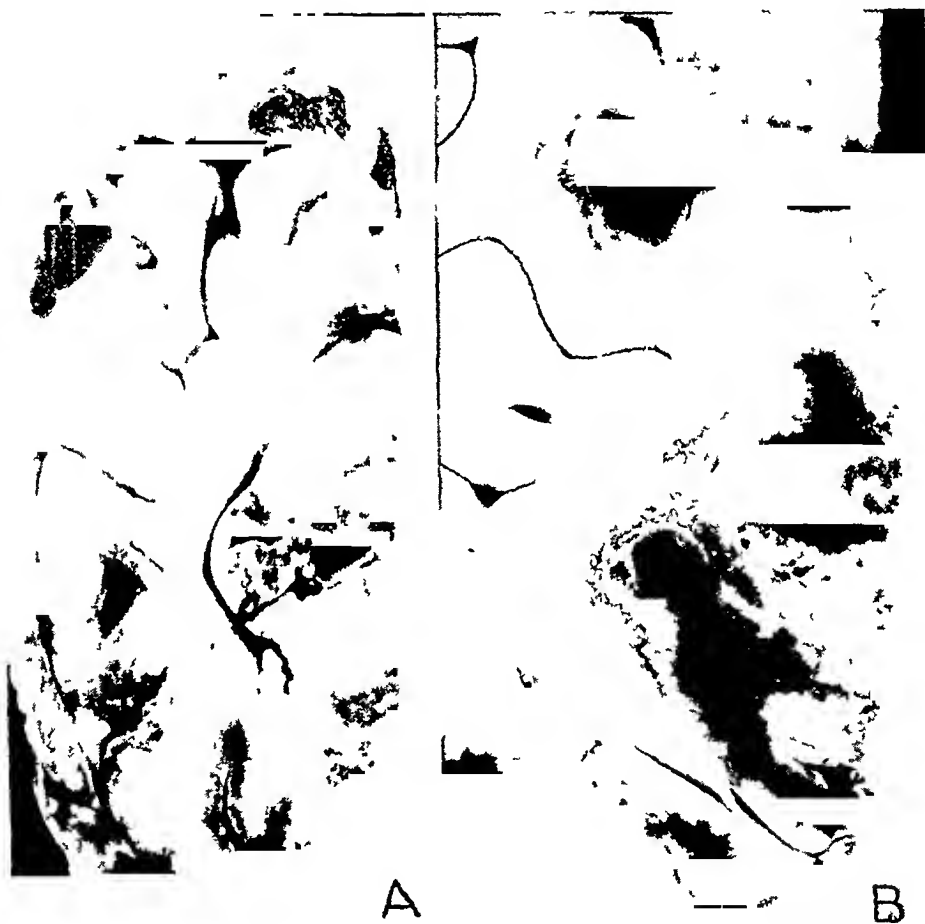


Fig. 4—*A*, cortical hemorrhage and subcortical softening in case 1. *B*, diffuse and discrete cortical hemorrhages in case 2, enlarged $\frac{2}{3}$.

impairment of the circulation resulting from the hemorrhagic infiltration of the cortex. The pressure of these accumulations of blood very likely interferes with the flow of arterial blood into the underlying white substance. The reddish coloration is due to the presence of myriads of small petechial hemorrhages, which may become confluent and thus not be clearly distinguishable from one another by the naked eye. In the subcortex the hemorrhages are often larger and more discrete.

The late appearance of this lesion is as yet unknown. Judging from the changes apparent after twelve days, one concludes that a more or less extensive atrophy and scarring of the cortex in the affected region will result. The scar will probably be a vascular one, as suggested by the tendency even in recent lesions to the formation of new blood vessels.

Histology—In the first few days there is little to be seen except the disorganized cortex with the infiltration of red blood cells. One outstanding change and one which persists for days is the remarkable and widespread dilatation of the small blood vessels.

After several days have elapsed, evidences of cellular proliferation in the pia and arachnoid are observed with a resultant irregular thickening of these membranes. In the cortex the nerve cells begin to lose their coloration in routine stained preparations, specific methods show a loss of tigroid material and disintegration of the neurofibrils. Cells in the vicinity of the hemorrhagic areas show more serious evidence of injury than do those more remotely situated. The microglia show transitional forms. The endothelial cells of the blood vessels also show early evidence of proliferation.

After an interval of nine days (case 1) more advanced changes are observed. The pia-arachnoid has become a definitely thickened structure owing to the proliferation of spindle-shaped cells and to its infiltration with leukocytes. About some of the larger pial and cortical blood vessels may be found large collections of lymphocytes. Large numbers of leukocytes are found scattered throughout the cortex, presumably the result of the hemorrhage. The cortex shows an advanced disturbance of the normal cyto-architectonics incident to hemorrhage and softening. The nerve cells in the seriously affected areas appear largely as "ghost cells." Tigroid pigment is absent from their cytoplasm. The neurofibrils show extensive disintegration when stained by the reduced silver method of Cajal, some cells being entirely devoid of argentophilic material, others having local collections of small perinuclear granules. About the areas of hemorrhage both in the cortex and subcortex this method shows the nerve fibers to be intensely impregnated with silver (preservation necrosis of Cajal). End-bulbs may be distinguished at times in these areas where the nerve fibers have been interrupted.

All transitional forms of microglia are shown by the combined method. The areas of cortex undergoing softening are occupied with compound granular corpuscles loaded with blood pigment, free fat or granules of argentophilic material, depending on the preparation studied. Other forms of interstitial elements (oligodendroglia and neuroglia) are poorly demonstrated in these necrotic areas, many of them evidently undergoing necrobiotic change.

The smaller cortical blood vessels still show an intense congestion. In the margins of the necrotic areas are found groups of blood vessels which show active proliferation of their endothelium and the development of new vascular buds. With special methods the endothelial cells are found to contain fat and argentophilic granules.

In the underlying white substance routine stains show pale infarcted areas with altered nerve fibers crossing them. These are evidently the result of an impairment of circulation.

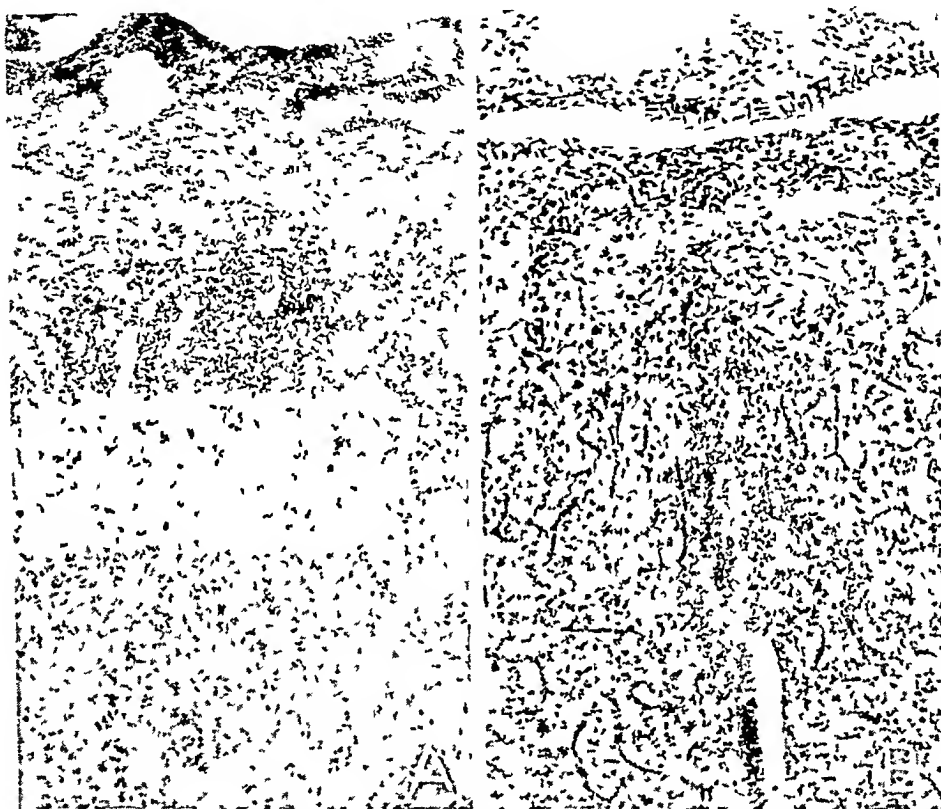


Fig 5—*A*, cortical and meningeal hemorrhage in case 1, hematoxylin and eosin, $\times 32$. *B*, thickening of the pia mater and perivascular hemorrhage, combined method, $\times 32$.

No opportunity has been offered to make a histologic study of the residual lesion after a period of months or years. One suspects that a vascular scar will result in the softened areas and permanent disorganization of the cortex as a result of the many areas of hemorrhage.

SUMMARY

Contusions of the occipital lobe are relatively uncommon. With the exception of those due to depressed fractures they assume certain characteristics which are of interest in the study of traumatic intracranial

lesions Diffuse contusion of the occipital lobe, characterized by a diffuse reddish coloration and softening of the affected cortex without gross morphologic disorganization, results from two types of mechanical disturbance The lesion may be primary and acute, resulting from forcing of the occipital cortex against the falx or tentorium when the head in motion strikes some relatively immovable object The force of the blow is expended on the sides or top of the head Although in the primary type the contusion of the brain is usually found on the same side as that of the injury to the scalp, it occurs on the opposite side of the occipital lobe and is therefore a contrecoup The secondary type is the result of local pressure by an expanding lesion such as edema or subdural or intracerebral hemorrhage In this type there is no necessary relationship between the side of the original injury and that of the contusion In any case the lesion is essentially a diffuse hemorrhagic softening of the cortex resulting from a rupture of small cortical veins incident to the sudden reversal of current in these vessels in the primary type, or to persistent and increasing obstruction by continued pressure in the secondary type Microscopically, the cortical and subcortical tissues are found to be infiltrated with blood The softening and disintegrating tissues are ultimately filled with compound granular corpuscles The appearance of the ultimate lesion is unknown since no example has as yet been studied

LIPIDS IN THE LIVER OF THE CAT DURING BILE STASIS AND BILIARY DECOMPRESSION

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HAROLD L STEWART, M D

AND

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PHILADELPHIA

There have been relatively few systematic studies of the effect of bile stasis on the quantity and distribution of lipids in the hepatic and Kupffer cells, and we have not found any reference to the effect of biliary decompression in this connection. In the course of studies of morphologic and functional changes occurring during experimental ligation of the common duct in cats, with subsequent release of obstruction, material was obtained suitable for a systematic study of this problem.

MATERIALS AND METHODS

Observations were made on thirty-nine cats during total bile stasis of from ten hours to forty-two days' duration and on twenty-one following biliary decompression of from one hour to seven days' duration. The animals were maintained on a diet of fresh scrap meat and milk. Comparative studies were made during stasis and decompression in the same animal in eleven instances. The animals were etherized, and the abdomen of each was clipped and washed with Harrington's solution, followed by alcohol. A midline incision was made, and the common duct was isolated. In some cases the duct was doubly ligated with linen thread and severed between the ligatures. In those animals which were to be subsequently subjected to decompression the duct was ligated close to the duodenum with linen tape 3 mm wide tied in a double surgical knot. After varying periods of stasis the ligature was carefully removed. Only those animals are included in this report in which the contents of the ducts promptly filled the segment distal to the point of ligation. The animals were subsequently killed under light ether anesthesia at varying intervals following release of the obstruction. In a few cases death occurred spontaneously, and material from these animals was rejected if evidence of postmortem change was detected.

All cases were excluded in which the duct system was not perfectly patent and in which bile could not readily be expressed through the ampulla of Vater. In this way we have been able to time the duration of total stasis and of decompression with a reasonable degree of accuracy. In eleven cases biopsy specimens were obtained at the time of removal of the obstructing ligature for the purpose

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Aided by the Jefferson Medical College Hospital Tumor Clinic and the Martin Research Fund.

of comparing the lesions of stasis with those of decompression in the same animals. All animals are excluded from the present consideration in which complicating factors were found to exist, among these were (1) reconstruction of the common duct or the presence of accessory ducts during the period of supposed stasis, (2) infection, (3) spontaneous or traumatic rupture of the duct and (4) nonpatency of the duct system following removal of the ligature. In cats 63 and 84, which are included in this report, biliary decompression was accompanied by acute suppression of bile formation.

Specimens were fixed in diluted solution of formaldehyde, U S P (1:10). Some were frozen, sectioned and mounted either unstained or stained with Nile blue sulphate, scarlet red, Fischler's¹ stain, dilute eosin and methylene blue, others were blocked in paraffin, cut and stained with hematoxylin and eosin and Mallory's and Van Gieson's connective tissue stains. Frozen sections (from 8 to 25 microns thick) were examined under crossed Nicol prisms for the presence of doubly refractile material.

EXPERIMENTAL OBSERVATIONS

Bile Stasis—The findings in the thirty-nine animals in this group are presented in detail in table 1. The quantity of stainable lipid in both hepatic and Kupffer cells was extremely variable during the first seven days of stasis. It was absent from the hepatic cells in only two instances (147 and 119) and from the Kupffer cells in only one (146) during this period. With stasis of increasing duration the quantity of stainable lipid decreased rather abruptly and was practically absent in animals with stasis of from 15 to 42 days' duration.

There was likewise wide variation in the distribution of lipid in different portions of the lobules in different cases, this variation was apparently unrelated to the duration of stasis within the first seven days. Moreover, there was no consistent relationship between the quantity and distribution of stainable lipids in the hepatic cells and their quantity and distribution in the Kupffer cells during this period.

During stasis, after the first few days, this material, even when present in large amount, occurred in the form of small granules. In the normal cat stainable lipids, which are usually present in abundance, tend to occur as droplets or globules of larger size. With the Nile blue sulphate stain the greater part of the stainable material was blue, however the subcapsular zone frequently contained pink or blue-pink granules, and occasionally variation in staining reaction was observed in different cells irrespective of their location.

In sections stained with hematoxylin and eosin vacuoles were noted which were not due to the presence of stainable lipid, as demonstrated by examination of frozen sections. In some cases they were found to be associated with doubly refractile, nonstainable material, in others they were due to the presence of glycogen and in some instances their identity could not be demonstrated.

¹ Mallory, F. B. and Wright, J. H. *Pathological Technique* ed 8, Philadelphia, W. B. Saunders Company 1924 p. 185.

Doubly refractile material was present in abundance in the Kupffer cells throughout all periods of stasis from ten hours to forty-two days. Except in a few cases of stasis of seven days' duration, much smaller quantities were noted in the hepatic cells. There was apparently no consistent relationship between the quantity and distribution of

TABLE 1—Results of a Study of the Effect of Bile Stasis on the Quantity and Distribution of Lipids in the Kupffer and Hepatic Cells

Cat	Weight, Gm	Days of Stasis	Stainable Lipid		Doubly Refractile Material	
			Kupffer Cells	Hepatic Cells	Kupffer Cells	Hepatic Cells
97	?	10 hrs	+	++	++++	+
94	?	16 hrs	++	+++	++++	++
69	?	20 hrs	++	++++	++++	+
70	?	1	+	+	+++	±
144	1,340	2	+++	++	+++	+
143	3,700	2	++++	++	++++	++
147	3,800	2	++	0	+++	±
154	1,370	6	+	+	++	+
126	442	6	+++	±	++	±
119	3,090	6	+++	0	+++	±
146	1,630	6	0	—	++	+
145	2,610	6	+	+	++	+
151	740	7	+	+++	+++	+++
114	2,580	7	+	+++	+++	++
152	4,800	7	++++	+++	+++	+++
150	1,180	7	++	++	+++	—
113	1,130	7	+	+	++	—
104	Kitten	7	±	+	+++	±
121	3,130	8	±	±	++	±
120	4,510	8	0	0	++	+
103	Adult	8	0	—	+++	±
28	Kitten	8	+	+	+++	±
102	Adult	8	0	0	+++	+
155	2,300	10	+	+	+++	++
123	3,160	10	±	±	++	±
101	Adult	10	+	—	+++	+
116	3,670	12	+	++	+++	+
25	Kitten	13	±	0	++	±
24	Kitten	15	0	0	+++	+
26	Kitten	15	0	0	+++	—
14	Adult	17	0	0	+++	+
50	Adult	18	+	+	++++	±
47	Kitten	20	±	±	++	0
58	Adult	22	0	0	++	±
60	Adult	25	0	0	++	±
51	Adult	27	0	0	++++	++
15	Adult	28	0	±	+++	±
54	Adult	30	0	0	+++	+
53	Adult	42	0	0	++	±

doubly refractile material and the quantity and distribution of stainable lipid. Frequently, the former was present in situations in which the latter was absent, on the other hand, at times the two were so intimately associated that the doubly refractile crystals produced marked irregularity in the outline of the stainable droplets and granules. In some instances these crystals occupied the central portions of the lipid droplets and granules, producing a vacuolated appearance in sections stained with Nile blue sulphate (fig. 1). Comparison of stained and unstained

frozen sections revealed an occasional discrepancy in the quantity of doubly refractile material. The reason for this is not apparent, since there was no consistency in this discrepancy. The tabulation of our observations is based on those sections showing the largest amount of this material in each case.

In stained and unstained frozen sections crystalline material was observed, the exact nature of which has not been determined. In Nile

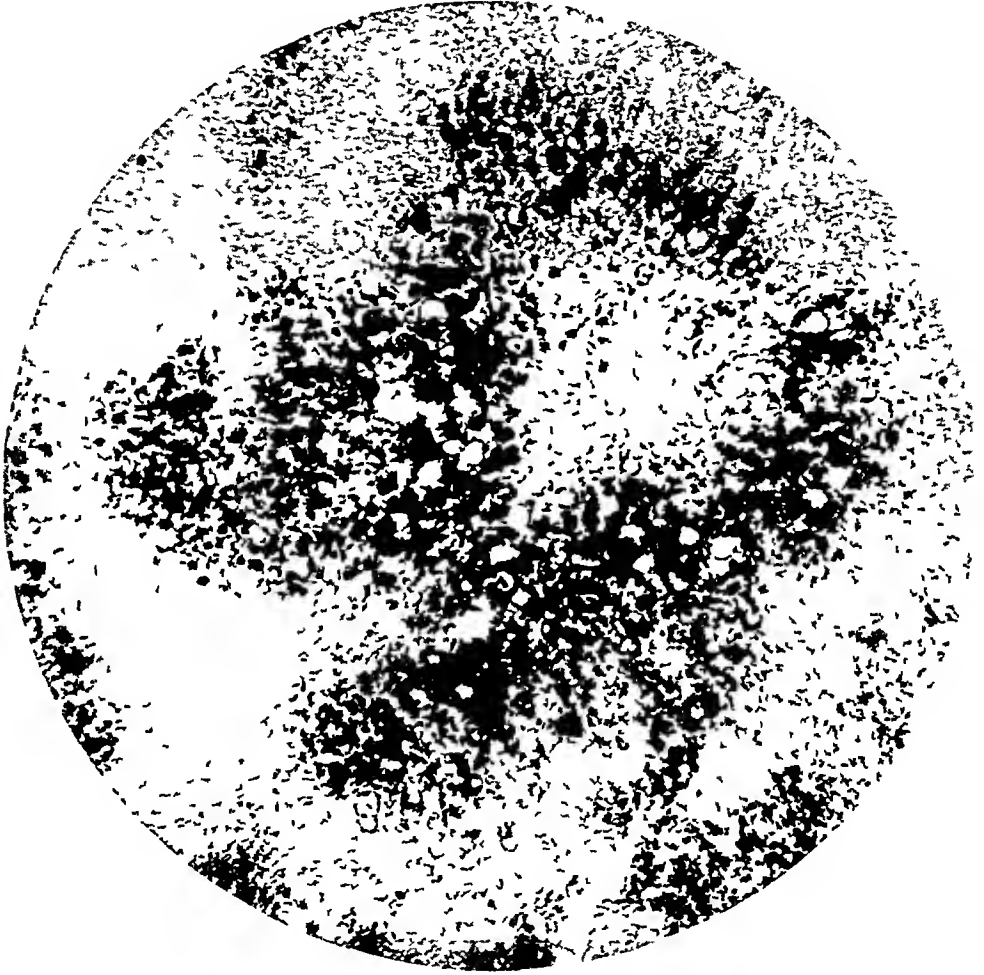


Fig 1 (cat 94) —Stasis sixteen hours. Liver shows vacuoles in stained lipid droplets, due to the presence of doubly refractile material. Frozen section stained with Nile blue sulphate, about $\times 100$.

blue sulphate preparations these appeared as needle-shaped or rod-shaped coarse crystals occurring singly or arranged in sheaves. They were noted as early as the sixteenth hour of stasis but were much more numerous and occurred more constantly in the later stages. These crystals were distributed irregularly throughout the lobule in patchy areas, being much more abundant in some lobules than in others. Within the lobule they appeared to be contained largely within Kupfer

cells, although the possibility that they were free within the sinusoids could not be definitely excluded. However, the fact that frequently large quantities of doubly refractile material in this situation extended to and stopped short at the central vein, none being present within the lumen of the latter, strongly suggests that these crystals were not free within the sinusoids. They were rarely observed in hepatic cells but were particularly numerous about and within the thickened portal radicles, in which situation they appeared in tissue crevices and within phagocytic cells. Similar crystals were seen in some cases lying free in the larger blood vessels where the serum had separated from the formed elements.

The great majority of these crystals were doubly refractile when examined under crossed Nicol prisms. In an attempt to establish their identity as fatty acids, Fischler's stain was applied, with inconclusive results. Many of the larger crystalline masses took the hematoxylin stain to some extent but also acquired a reddish cast, in some cases small, sharply defined black crystals could be seen in intimate association with those mentioned.

The stainable lipids, doubly refractile material and brownish crystals considered in the foregoing paragraphs bore no apparent relation to the degenerative and necrotic lesions occurring in the liver in the presence of bile stasis as described elsewhere.¹¹ It is difficult to determine the presence or absence of any relationship between the quantity of stainable lipid and the sporadic degenerative and necrotic lesions during the first week of stasis, this is due chiefly to the coincidental abundance of lipids and the activity of degeneration and necrosis in the inner portion and sporadically throughout the lobule during this period. However, in the later stages extensive degenerative changes could be seen in cases in which stainable lipid was absent. Nevertheless, it is possible, of course, that the stainable lipid granules occasionally observed during the late period of stasis may be associated with the degenerative process in the hepatic cells.

The areas of focal necrosis present during the first thirteen days of stasis usually contained many small lipid granules within the shadowy outlines of necrotic and disintegrating hepatic cells which, together with the latter, were phagocytosed by macrophages. Lipid in this situation occurred independently of the presence or absence of stainable lipid in the remainder of the lobule. Doubly refractile crystals were rarely observed in areas of focal necrosis even when present elsewhere in the lobule.

¹¹ Stewart, H. L., and Lieber, M. M. *Arch. Path.* **19**: 34, 1935. Cantarow, A., and Stewart, H. L. *Am. J. Path.* **11**: 561, 1935.

The areas of hyaline necrosis beneath the capsule of the liver and about the portal radicles frequently contained dioplets of stainable lipid occurring both within necrotic hepatic cells and within macrophages, which were apparently actively engaged in phagocytosing and removing the necrotic material. In the greatly thickened portal radicles resulting from the organization of hyaline necroses there were frequently collections of macrophages loaded with fat in immediate relation to the large bile ducts which were being destroyed. Stainable lipids were also present in the necrotic epithelial cells lining these ducts. We have not seen stainable lipid in the epithelial cells of the bile ducts during

TABLE 2—*The Quantity and Distribution of Lipids in the Kupffer and Hepatic Cells Following Decompression*

Cat	Weight, Gm	Days of Stasis	Days of Decompression	Stainable Lipid		Doubly Refractile Material	
				Kupffer Cells	Hepatic Cells	Kupffer Cells	Hepatic Cells
132	3,800	1	1	+++	+	++	±
131	3,230	1	1	+	+++	++	+++
147*	3,800	2	1	++	0	+++	±
143*	3,700	2	1	++++	+	++++	+
144*	1,340	2	1	+	++	+++	---
87	3,180	4	7	+	++	++	+
98	2,250	5	1	+	+++	+++	---
65	1,950	6	3	++	++	+++	+
86	2,400	7	1	+	+++	++	---
115*	1,130	7	4 hrs	++	+++	+++	—
114*	2,580	7	1	++	+	+++	++
150*	1,180	7	1	±	+++	++	++
152*	4,800	7	3	+++	—	+	+++
120*	4,150	8	2 hrs	+	+	+++	—
121*	3,130	8	1	±	±	++	++
123*	3,160	10	1 hr	±	±	++	—
122	4,280	11	1	+++	0	+++	±
64	1,950	11	1	+	++	+++	—
116*	3,670	12	1	+	+	++	+
128	2,530	15	2	++++	±	+++	—
125	2,560	16	3	++++	±	++++	+

* Observed during stasis also (table 1)

stasis except when the ducts were undergoing destruction as a result of the organization of areas of hyaline necrosis

Biliary Decompression—The findings in the twenty-one animals in this group are presented in detail in table 2. The quantity of stainable lipid in both hepatic and Kupffer cells was extremely variable during all periods of decompression from one hour to seven days following total stasis of from one to sixteen days' duration. It was present in the Kupffer cells in every case and was absent from the hepatic cells in only two instances (decompression one day, stasis two and eleven days). The observations made during stasis regarding the variation in the distribution of stainable lipids and the absence of any consistent relationship between the quantity in the hepatic cells and that in the Kupffer cells were also true during decompression. Although occasion-

ally there seemed to be a distinct tendency toward droplet formation during decompression, in general the lipid material presented much the same appearance as during total stasis

Comparative studies during stasis and decompression in eleven cases revealed nothing of particular significance except in cat 120, in which no stainable lipid was present in the hepatic parenchyma at the end of eight days of stasis whereas stainable lipid was observed in both hepatic and Kupffer cells two hours after release of the obstruc-

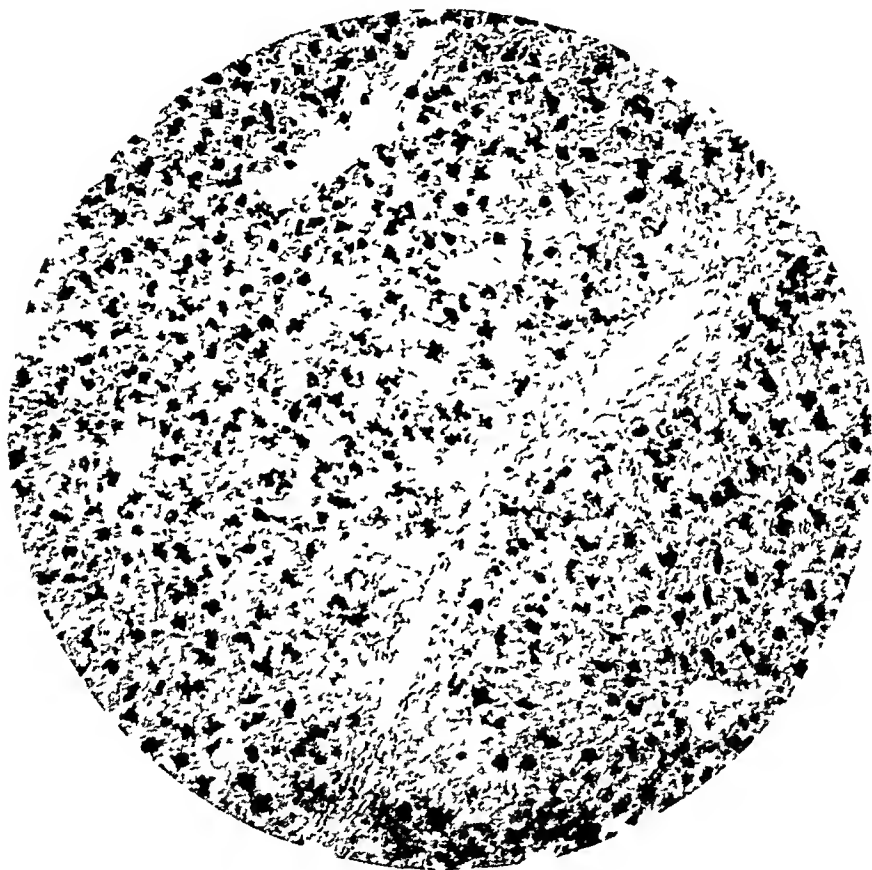


Fig 2 (cat 128) —Stasis fifteen days, decompression two days Liver shows large amounts of stainable lipid in Kupffer cells Frozen section stained with Nile blue sulphate, about $\times 60$

tion No change occurred in three animals, and a variable change was noted in the remainder, the nature of which may be observed by comparing the findings recorded in table 1 and table 2 Of particular interest is the fact that large amounts of stainable lipid were present in the Kupffer cells in cats 128 and 125 two and three days, respectively, after periods of bile stasis of fifteen and sixteen days' duration, at which time stainable lipid is almost invariably absent or present in only very small quantity (fig 2) Doubly refractile material

was present in every case and differed in no essential respect from that observed during total stasis. Comparative studies in individual cases revealed relatively insignificant differences, which may be observed by referring to data presented in tables 1 and 2.

A peculiar lesion was noted in cat 132 twenty-four hours following relief of stasis of twenty-four hours' duration (figs 3 and 4). Large

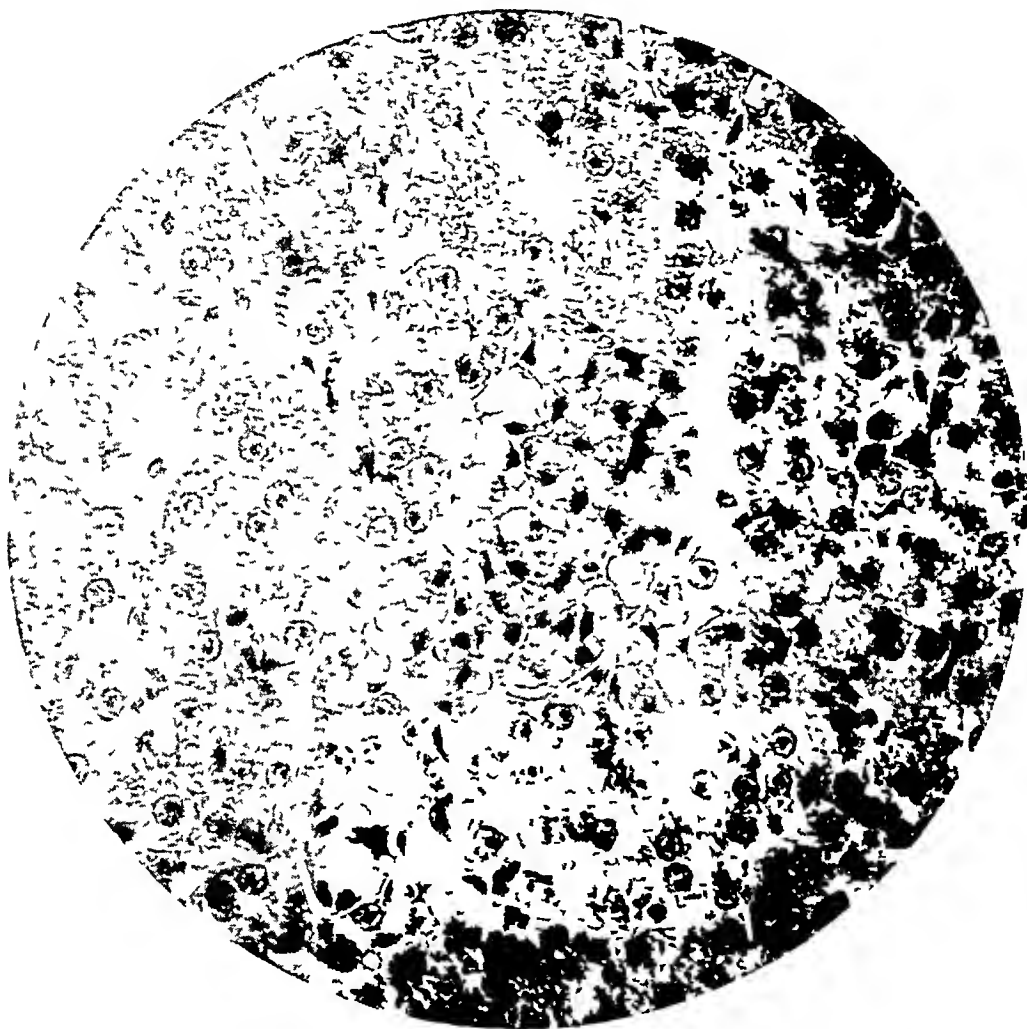


Fig. 3 (cat 132) —Stasis one day, decompression one day. Liver shows large sharply demarcated nodular lesion composed of vacuolated pigmented cells, apparently hepatic cells. Note two smaller nodules below and to the left of the large one. Hematoxylin and eosin, about $\times 450$.

vacuolated deeply pigmented cells, apparently hepatic cells, were scattered throughout the liver and frequently were collected in groups of as many as thirty-five cells. The line of demarcation from the surrounding parenchyma was sharp and often consisted of a canaliculus. These lesions, which occurred indiscriminately in all parts of the lobule, were

regarded as probably localized areas of degeneration, the pigmentation being accentuated by the degenerated and vacuolated cytoplasmic background. These lesions contained large amounts of stainable lipid which stood out in sharp contrast to the hepatic cells in the remainder of the parenchyma which were almost devoid of stainable fat. On the other hand, these areas contained few or no doubly refractile crystals although

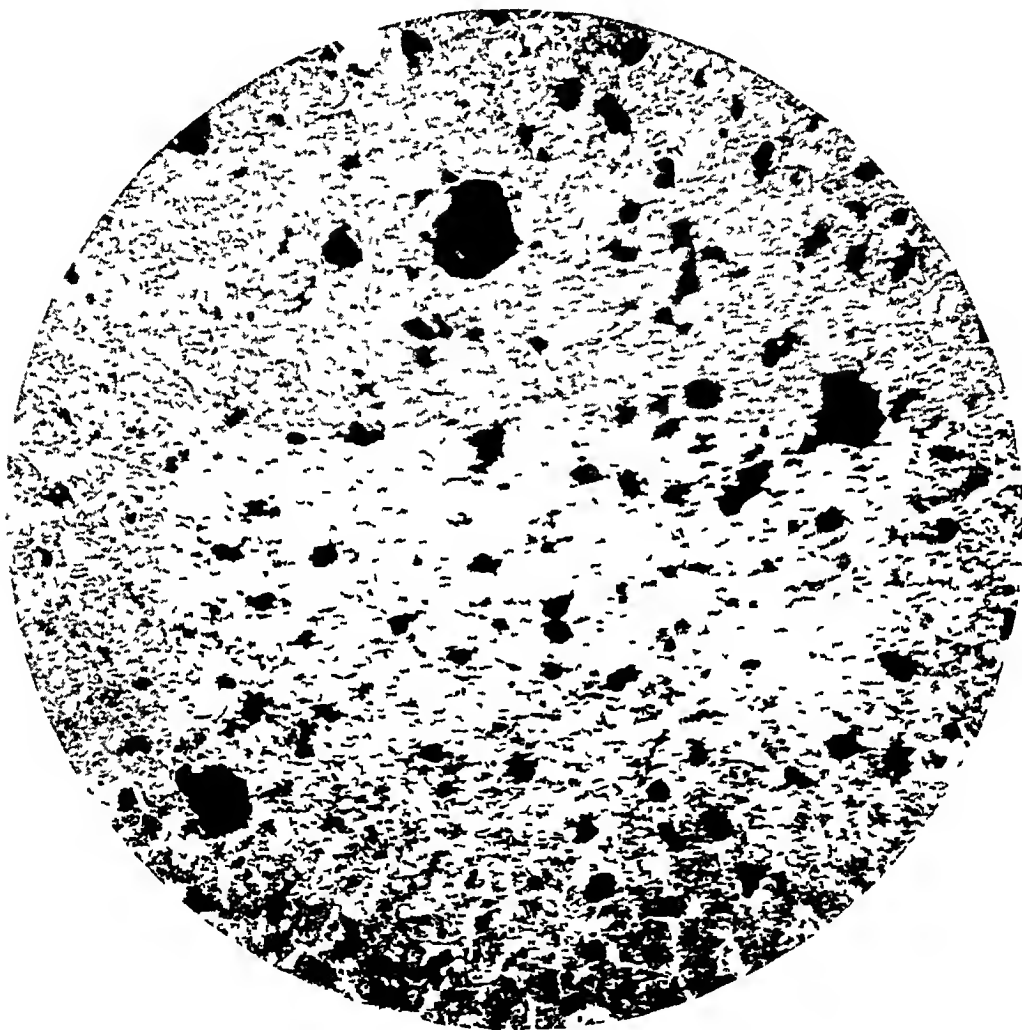


Fig 4 (cat 132)—Stasis one day, decompression one day. This shows the high lipid content of the nodular lesions illustrated in figure 3. Note the relative absence of stainable lipid in the hepatic cells and the large amount in the Kupffer cells. Frozen section stained with Nile blue sulphate, about $\times 200$.

the Kupffer cells in the surrounding parenchyma contained relatively large quantities of such crystals.

COMMENT

The literature contains few studies of the effect of total bile stasis on the quantity and distribution of lipids in the liver, and no reference

could be found to studies of the effect of decompression in this connection Mayer,² who is generally regarded as the first to study systematically the morphologic changes in the liver of the cat following ligation of the common duct, concluded from observations on four animals that fatty degeneration of the liver does not occur as a result of bile stasis. However, all of these animals showed peritonitis at autopsy. These findings were in direct contrast to those of Leyden³ who, studying twenty dogs with total stasis of from one to thirty-three days' duration, reported an increase in hepatic fat during the period of stasis. All but one of these animals died of peritonitis. The work of Legg⁴ is of distinct significance because of the relatively large number of animals employed (sixteen cats), the detailed report of the morphologic findings and the infrequency of gross infection despite the fact that aseptic technic was not employed. With the exception of one case in which large amounts of fat were present at the end of seven days of stasis, Legg found practically no fat droplets in the liver after the fourth day of stasis in the absence of spontaneous reconstruction of the common duct. His conclusions were as follows:

As to the infiltration of the cells with oil, it is difficult to determine whether it is an accident or the result of the operation. The evidence would incline, I think, to the former. In those animals who lived into the second or third weeks, the large fat drops in the liver cells were no longer seen. They were replaced by granules, colorless and insoluble in acetic acid, probably therefore fat. This granular state of the cells may be looked upon as part of the atrophy caused by the growth of the connective tissue, just as in ordinary cirrhosis.

This problem has apparently never been subjected to systematic and detailed investigation, but the occasionally reported observations, usually incidental and sporadic, are summarized in the statement by Ogata⁵ that with obstruction of the common duct hepatic fat is markedly reduced in almost all animals (guinea-pig, rat, rabbit, dog, mouse, frog, pigeon), these findings coincide with observations made on human beings. Ogata found fat in the hepatic cells during prolonged stasis in only one dog (thirty-five days) which was very obese.

Our findings in the present series are in accord with the earlier observations regarding the decrease in the stainable lipids of the liver in the later stages of stasis. It is interesting to note that although there was rather wide variation in the fat content of both hepatic and Kupffer cells during the first week of stasis a sudden marked decrease occurred at about the seventh day, even small quantities of stainable lipid being

² Mayer, H. *Med. Jahrb.*, 1872, p. 133.

³ Leyden, E. *Beitrage zur Pathologie des Icterus*, Berlin, A. Hirschwald, 1866, p. 83.

⁴ Legg, W. *St. Barth. Hosp. Rep.* 9:161, 1873.

⁵ Ogata, T. *Beitr. z. path. Anat. u. z. allg. Path.* 55:236, 1913.

seldom found after that time. There are few data available in the literature regarding the distribution of fat between the hepatic and Kupffer cells during total bile stasis. Although there was no consistent relationship between the quantities of stainable lipid in these two situations during the first week of stasis, the fact that it diminished simultaneously in both types of cells after that time is probably of significance.

The cause of the marked decrease in stainable lipid is not readily apparent. It is probably not dependent on impaired nutrition which, according to most investigators, is usually accompanied by an increase in hepatic fat. Furthermore, although these animals did not take a normal amount of food, they almost invariably ate some of the food offered them and no relationship could be demonstrated between the state of nutrition and the fat content of the liver. The fact that in the first week of stasis there was no consistency between the distribution of fat and that of sporadic degenerative changes within the lobules, coupled with the fact that in the later stages fat was frequently absent from cells which were markedly degenerated, suggests that this variation in the amount of stainable lipid is not dependent directly on regressive changes in the cells. In the focal midzonal and hyaline necroses, however, the contained fat was at times obviously associated with the necrotic process, since the adjacent parenchyma was often free from fat. It is probable that the observed changes in the stainable lipids of the hepatic and Kupffer cells are dependent on some alteration in the metabolic activity of these cells incident to the state of total bile stasis. This hypothesis is perhaps supported by the fact that relatively large quantities of fat were present in animals (128, 125) two and three days after the relief of stasis of fifteen and sixteen days' duration, on the basis of our observations during total stasis, it is highly improbable that the hepatic and Kupffer cells contained even a small amount of stainable lipid at the time of the release of the obstructing ligature.

In view of the early studies of hepatic fat in bile stasis, which was almost invariably complicated by infection, the findings in certain of our cases in which suppurative cholangitis was present at autopsy are of interest. These cases are not included in the material presented in the tables. A few animals with this condition were found to have rather large quantities of stainable lipid in the hepatic and Kupffer cells, particularly in the former, in the late stages of stasis, differing markedly in this respect from the animals in which the last stages of stasis were uncomplicated. A moderately large quantity of stainable lipid was observed in the Kupffer cells in one animal with stasis of twenty-nine days' duration, this animal, however, had been rendered acutely anemic by repeated withdrawal of large amounts of blood by cardiac puncture, which may have been responsible for the unusually large quantity of lipid present.

Double refraction of lipid material is indicative of the presence of large quantities of cholesterol and cholesterol esters in the lipid mixture. The apparent lack of quantitative relationship between the doubly refractile material and the stainable lipid in the Kupffer cells during total stasis suggests that the factors underlying the metabolism of these two classes of substances in the liver are essentially different. This difference was much more pronounced in the Kupffer than in the hepatic cells, in fact, in a few instances, particularly with stasis of seven days' duration, a relatively large quantity of stainable lipid in the hepatic cells was associated with a similarly large amount of doubly refractile material. This suggestive metabolic difference is further supported by the lack of consistent relationship in the distribution of stainable lipid and doubly refractile material in the hepatic cells.

Levine⁶ found doubly refractile lipid substances consistently in the hepatic cells of twenty-seven human subjects who died of accidental causes, but was unable to demonstrate any in the Kupffer cells. On the basis of the observation of Anitschkow⁷ and others that these cells store cholesterol and cholesterol esters removed from the blood stream, Levine stated that their absence in this situation under essentially normal conditions indicates that the cholesterol is probably passed to the hepatic cells too rapidly to be demonstrated in the Kupffer cells by the technic employed. However we were able to demonstrate large quantities of doubly refractile lipid material in the Kupffer cells of normal animals studied in conjunction with the present experimental series. Nevertheless, the presence of consistently large amounts in the Kupffer cells and small amounts in the hepatic cells during total stasis suggests that the transfer of this material from the former to the latter is considerably impaired under these conditions and that some degree of impairment in this connection persists during decompression of one hour to seven days' duration.

SUMMARY

Studies were made of the quantity and distribution of stainable lipid and doubly refractile material in the liver in thirty-nine cats with uncomplicated total bile stasis of from ten hours to forty-two days duration and in twenty-one cats following biliary decompression of from one hour to seven days' duration.

The quantity of stainable lipid decreased markedly in both hepatic and Kupffer cells during stasis and was practically absent in the majority of animals after the seventh day. A return of large quantities of stain-

⁶ Levine, V. Arch Path **14** 345, 1932

⁷ Anitschkow, N. N. Deutsche med Wchnschr **39** 741, 1913

able lipid was noted in animals several days after relief from prolonged total stasis

Doubly refractile material was present in the Kupffer cells in large quantities throughout the entire period of total stasis differing markedly from the stainable lipid in this respect

Although necrotic lesions frequently contained large quantities of fat, our observations during the early and late stages of stasis indicate that the stainable lipids distributed indiscriminately throughout the lobule are not necessarily dependent on regressive changes in the affected cells

Stainable lipid was not observed in the epithelium of the bile ducts except in the later period of stasis at a time when the ducts were undergoing destruction as a result of organization of areas of hyaline necrosis

It is suggested that the consistently large amount of doubly refractile material in the Kupffer cells and the relatively small quantity in the hepatic cells during stasis are dependent on a delay in the transfer of this material from the former to the latter under the experimental conditions

RENAL DENERVATION

EFFECT OF DAILY INJECTIONS OF COLON BACILLI AND PITRESSIN
ON THE DENERVATED KIDNEY OF THE DOG

GEORGE MILLES, M D

CHICAGO

AND

MAURICE HARDGROVE, M D

MILWAUKEE

In previous experiments¹ on dogs in which unilateral denervation of the kidney had been performed one of us (G M) demonstrated by means of roentgenograms taken after injection of a suspension of bismuth oxychloride into the renal vascular bed that

1 There is dilatation of the renal vascular bed after denervation

2 The vascular bed of the denervated kidney does not contract after the intra-arterial injection of epinephrine, indicating the completeness of the denervation

3 The denervated kidney is not damaged by repeated chilling as is the normal kidney

4 A single injection of a toxic agent, such as snake venom causes greater changes in the denervated than in the normal kidney

Of that series of twenty-six dogs killed from one to six months after renal denervation, the majority displayed an increase in the size of the denervated kidney, in a few the denervated kidney was slightly smaller, and in one the organ on which operation was performed was definitely contracted

On the basis of these observations further experiments were undertaken to determine the response of the denervated kidney when exposed to various agents over long periods. Unilateral renal denervation through the lumbar route was carried out in a group of dogs, and a period of approximately two weeks was allowed for complete recovery. The dogs were divided into three groups. Each animal of the first group received 1 cc of a two day broth culture of colon bacilli intravenously daily. No immediate effects were noted from the injections. The second group received 1 cc of pitressin intravenously daily. In most instances the injection was followed in a few minutes by vomiting and defecation and even with this dose some animals died. The

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine

1 Milles, G, Muller, E G, and Petersen, W F Arch Path **13** 233, 1932

third group received 1 cc each of the broth culture of colon bacilli and of pitressin intravenously. The immediate reaction of these animals to the injection were the same as those of the second group. The injections were made daily—those of pitressin with the idea of producing repeated vasoconstriction and those of the colon bacillus as a bacterial agent of rather low virulence capable of doing damage to tissues.

After from three to eight weeks the animals were killed and the kidneys weighed. Tissues were fixed in formaldehyde and stained with hematoxylin and eosin and with azocarmine and methylene blue² for microscopic study. Ten dogs of the series survived long enough to be of value in this report.

EXPERIMENTAL INVESTIGATION

Group 1—Daily intravenous injections of colon bacilli were made.

Dog 1—Denervation of the left kidney was performed on Feb 21, 1934, and the dog died on March 8.

The normal kidney weighed 40 Gm. Its capsule stripped readily, leaving a smooth, brownish-red surface. On the cut surface the cortex was of average width, grayish red and sharply demarcated from the medulla.

Microscopically the glomeruli were normal. The tubular epithelium was well preserved. The blood vessels were moderately dilated and contained a considerable number of pigment-filled macrophages. In a single area the normal structures had been replaced by dense hyalinized connective tissue, blending with a focus of round cell infiltration.

The denervated kidney weighed 25 Gm. Its capsule was thickened and gray and stripped with considerable resistance. The surface was pale gray to grayish pink. The cut surface was pale gray, the cortex was narrowed, and the medulla was poorly demarcated.

Microscopically the glomeruli were distended. Bowman's space was free from contents, and Bowman's capsule, which was composed of the usual flat cells, was in many instances surrounded by a zone of round cell infiltration, which usually extended into the interstitial tissue along the blood vessels. Moderate degenerative changes, chiefly in the form of cloudy swelling and occasional necrosis, were present in the tubular epithelium. There was moderate increase in the interstitial connective tissue. The blood vessels were markedly dilated and densely packed with red cells and pigment-filled macrophages. The smaller arterioles displayed moderate increase in the thickness of their walls and of the perivascular connective tissue.

Dog 2—The kidney was denervated on February 21, and the dog was killed on June 19.

The normal kidney weighed 40 Gm. It was normal in gross appearance, answering to the same description as that of the corresponding kidney of dog 1.

Microscopically the glomeruli were of moderate size, there was an increase in the number of their cellular elements, particularly of the interstitial cells. Bowman's space was free from contents, Bowman's capsule was composed of a single layer of flat cells. In an occasional glomerulus the basement membrane

² McGregor, L. *Am J Path* 6:347, 1930.

of Bowman's capsule was proliferated and hyalinized. In such instances the afferent vessel was thick-walled, its lumen was narrowed, and a more or less well defined mantle of round cells was seen about the glomerulus. The tubular epithelial cells were fairly well preserved, although rather small, and a considerable amount of granular debris was present in their lumens. The arterioles were moderately thick-walled, and many of them were surrounded by a mantle of round cells.

The denervated kidney weighed 40 Gm. The capsule was thickened; otherwise the kidney showed no marked gross difference from that on which no operation was performed.

Microscopically the glomeruli were larger and the capillaries more dilated, and varying degrees of interstitial hyalinization were present, otherwise the section was essentially the same as that taken from the kidney on which no operation was performed.

Dog 3—Denervation was performed on March 23, and the dog was killed on June 19.

The normal kidney weighed 35 Gm. The capsule was thin and stripped readily, leaving a smooth, dark brownish-red to purplish-red surface. On the cut surface the cortex was well preserved and dark brownish red and the markings were sharply defined. The medulla was clearly demarcated.

Microscopically the glomeruli were rather variable in size and appearance, some were large and cellular, and others were inconspicuous with small pyknotic nuclei and markedly increased and hyalinized interstitial tissue. In others there was loss of capillary tufts so that in some instances only a single atrophic tuft remained. Bowman's space contained a variable amount of homogeneous, pale-staining material, and Bowman's capsule varied from a single flat to a single swollen layer of cells, or to several layers of cells. The tubular epithelium was narrowed, the lumens of the tubules contained a considerable amount of granular debris. Here and there, an arteriole with a markedly thickened and hyalinized media and marked proliferation of perivascular connective tissue was seen. In such vessels the lumen was small. Except for the moderate degree of perivascular fibrosis there was no increase in the connective tissue.

The denervated kidney weighed 20 Gm. Its capsule was thick, gray and adherent. The surface was pale gray, and the consistency was markedly denser. On the cut surface the cortex was pale and narrow, and the medulla was poorly demarcated.

Microscopically (fig 1) the following picture was uniformly present. The glomerular tufts were small, cellular and generally fairly well preserved. Bowman's space was free from contents. There was moderate to marked proliferation of the basement membrane of Bowman's capsule. Around many glomeruli a narrow mantle of round cells was seen. The tubules showed marked degenerative changes: their cells were flat or entirely lost, and their lumens were filled with hyaline plugs. There was marked increase in the interstitial connective tissue, which replaced the tubules to a large extent. The arterioles were thick-walled and hyalinized, and vacuolation of the intimal and medial cells was present. The arterioles were surrounded by a mantle of proliferating connective tissue.

Dog 4—The kidney was denervated on April 26, and the dog was killed on June 21.

The normal kidney weighed 55 Gm. Grossly it appeared normal.

Microscopically an occasional glomerular tuft presented degeneration of a single loop, and in a few Bowman's spaces granular debris and red blood cells

were seen. The tubular epithelium was well preserved. The blood vessels were moderately distended with red cells.

The denervated kidney weighed 40 Gm. The capsule was thick, pale gray and markedly adherent. The renal parenchyma was pale gray to grayish pink.

Microscopically the glomerular tufts were compact and displayed rather marked cellular proliferation. Bowman's space was free from contents, and Bowman's capsule in many instances was thickened as a result of the proliferation of the



Fig 1 (dog 3)—Section of the denervated kidney after injections of a suspension of *B. coli* daily for three months. Hematoxylin and eosin stain, $\times 240$.

basement membrane. There was marked focal proliferation of the interstitial connective tissue, which radiated from the thick-walled arterioles. The lumen of the arterioles was diminished. The tubular epithelium was narrow and atrophic, and in the areas in which proliferation of interstitial connective tissue was most marked the tubules were entirely replaced. This section showed marked focal increase of connective tissue, which was especially well brought out by the azocarmine stain.

Dog 5—The operation was performed on May 1, and the dog was killed on June 21

The normal kidney weighed 33 Gm. Grossly the kidney appeared normal.

Microscopically the glomeruli were compact and cellular, and their capillary tufts were moderately dilated. The tubular epithelium was fairly well preserved. The blood vessels were normal in appearance, and there was no increase of interstitial connective tissue.

The denervated kidney weighed 37 Gm. The glomerular tufts were more markedly dilated and variable in their cellular content than those of the normal kidney. The tubular epithelium was well preserved. The blood vessels displayed no pronounced change except that they were moderately distended with red cells.

Summary of Group 1—The daily intravenous injection of a suspension of colon bacilli into the five dogs of this series in which unilateral renal denervation had been performed resulted, after a period of two months, in demonstrable proliferation of the media of the small arterioles and proliferation of the adventitial connective tissue of the vascular coat. There was an associated low grade inflammatory reaction with interstitial fibrosis and scarring, involving almost exclusively the denervated kidney in four of the animals. The denervated kidney was markedly decreased in size in three animals, increased in one and unchanged in the fifth.

Group 2—Daily intravenous injections of pitressin were given.

Dog 6—The animal appeared normal on April 3 and was killed on July 25.

The kidney on which operation had been performed weighed 40 Gm and appeared normal on gross examination.

Microscopically the glomerular tufts were large, and their capillaries were distended so that Bowman's space was present as a narrow slit in most instances. In an occasional glomerulus necrosis of a single loop was seen to have occurred, and red cells were present in Bowman's space. In other instances the glomerulus was smaller than its neighbors, and Bowman's capsule was thickened and hyalinized. The basement membrane of these glomeruli was proliferated and surrounded by a mantle of round cells. Adhesions between the capsule and the tufts were seen in a few instances. These changes were focal in their distribution. The arterioles were moderately thick-walled, and there was increase in the perivascular and interstitial connective tissue. The tubular epithelium was well preserved although the cells were somewhat smaller than normal, and a small amount of debris and a few hyaline plugs were seen in the lumen.

The denervated kidney weighed 22 Gm. The capsule was thick, pale gray and adherent. The cortex was narrow, very pale pinkish gray and poorly demarcated from the medulla.

Microscopically (figs 2 and 3) the tissue was the seat of profound changes characterized by diminution in the size of the various structures so that the glomeruli were arranged close together. The glomerular tufts displayed more or less marked atrophic changes, and in practically all the instances there was marked proliferation with more or less hyalinization of the basement membrane of Bowman's capsule. The tubules were almost entirely lost, having been replaced by markedly proliferated interstitial connective tissue. Their small lumens, particularly those of the collecting tubules, contained hyaline plugs. The arterioles

were markedly thickened, their lumens were markedly narrowed or completely obliterated, and there was marked proliferation of perivascular connective tissue as well as of the interstitial connective tissue already described, which was the seat of diffuse round cell infiltration

Dog 7—Denervation was performed on May 1, and the dog was killed on June 9

The normal kidney weighed 38 Gm Grossly it appeared normal

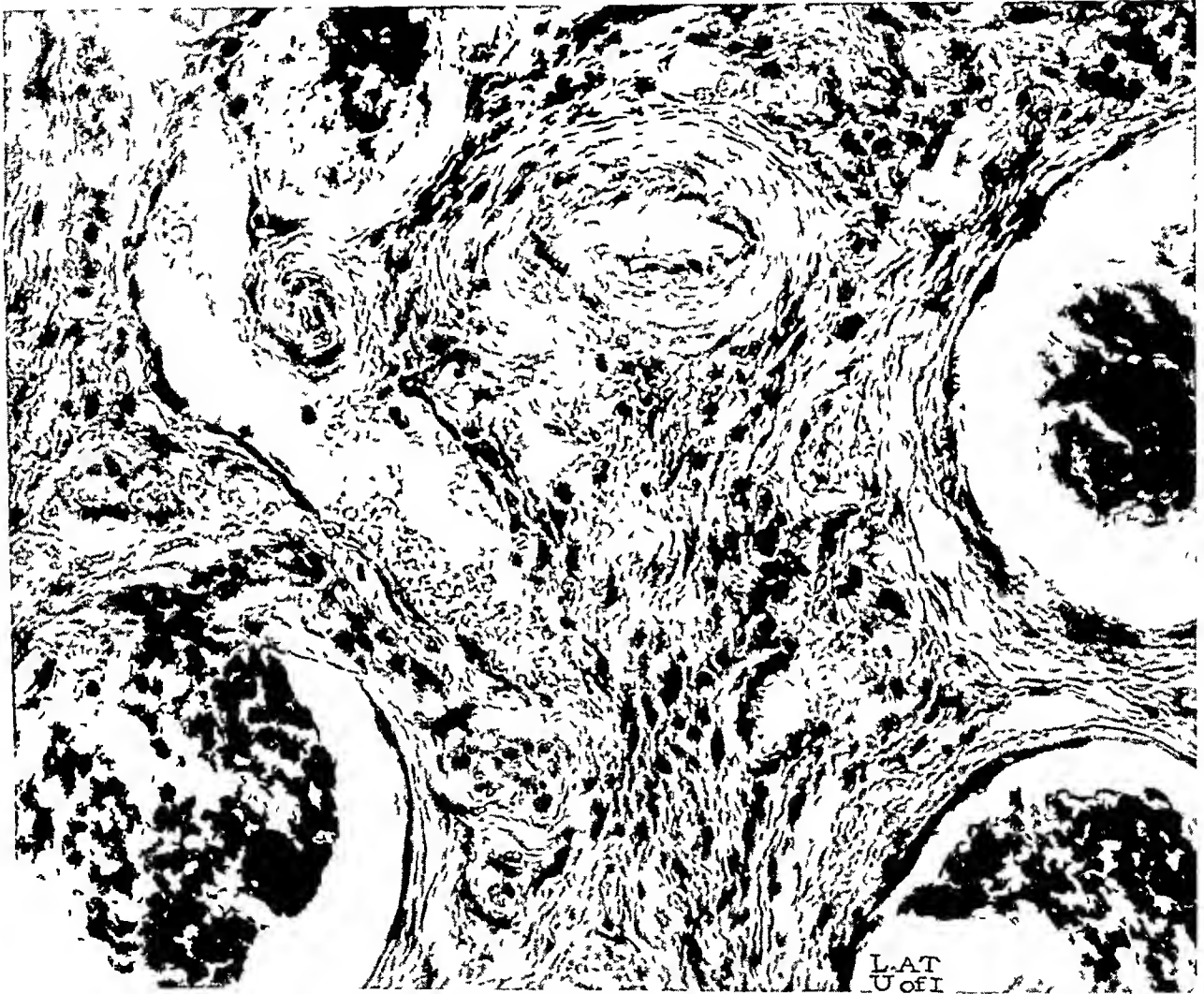


Fig 2 (dog 6)—Section of the denervated kidney after intravenous injections of 1 cc of pitressin daily for three months Hematoxylin and eosin stain, $\times 480$

Microscopically the glomeruli were distended, the capillaries were filled with red cells, and Bowman's space was free from contents Bowman's capsule was composed of a single layer of flat cells The tubular epithelial cells were granular, and their free margins were frayed There was a moderate degree of cellular desquamation into the lumens The larger blood vessels were distended with red cells

The denervated kidney weighed 38 Gm The capsule was slightly thickened and moderately adherent

Microscopically the glomeruli were essentially normal. The tubular epithelium was well preserved. The larger vessels were widely distended with red cells. There was no increase in perivascular or interstitial connective tissue in either section.

Dog 8—Unilateral nephrectomy was performed on April 26, and the dog was killed on May 31.

The kidney weighed 25 Gm. Grossly it appeared normal.

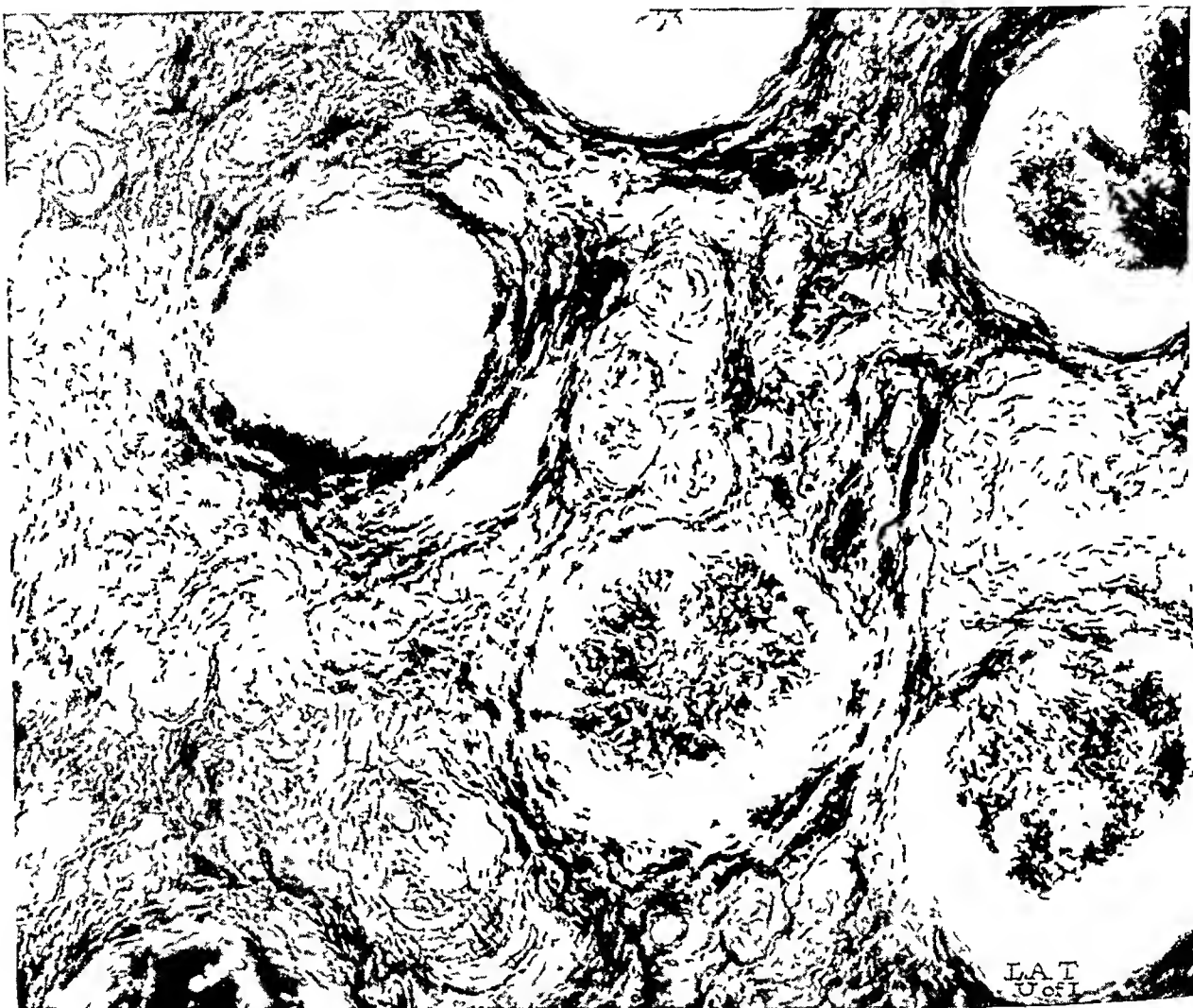


Fig 3 (dog 6)—Section of the denervated kidney after intravenous injections of 1 cc of pitressin daily for three months. Azocarmine and methylene blue, $\times 400$.

Microscopically the kidney showed moderate hyaline changes in the glomerular tufts, granular debris filling Bowman's spaces, marked edema and proliferation of the interstitial tissue widely separating the tubules. There were profound degenerative changes in the tubular epithelial elements. These epithelial cells were present as an amorphous or granular mass in which only a few nuclear shadows remained and the cellular boundaries were entirely lost. The smaller

arterioles were diminished in size, the intima was moderately proliferated the media was hyperplastic, and there appeared rather marked proliferation of the perivascular connective tissue

Summary of Group 2—Because of deaths after the injection of doses of pitressin larger than 1 cc and occasional deaths even with a dose of 1 cc of pitressin intravenously, only three dogs survived long enough for the experimental data to be used in this report. In the kidney of one dog pronounced perivascular and interstitial fibrosis, associated with marked round cell infiltration, similar to that seen in some of the dogs receiving colon bacilli, occurred. In the other dog the denervated kidney appeared to withstand the injections of pitressin with less resulting damage than did the kidney on which no operation was performed. In the unilaterally nephrectomized animal the remaining kidney was uninjured by a month of treatment.

Group 3—One cubic centimeter of pitressin and 1 cc of a suspension of colon bacilli were given daily.

Dog 9—Denervation was performed on June 27, and the animal was killed on September 5.

The normal kidney weighed 21 Gm. The capsule stripped readily. The surface was smooth and dark brownish red.

Microscopically the glomeruli were large, more or less completely filling Bowman's space. In focal areas radiating through the cortex from the surface toward the medulla were zones in which the interstitial connective tissue was proliferated and infiltrated with round cells. There was rather pronounced round cell infiltration of the periglomerular interstitial tissue. In these areas the tubular epithelium was narrow and atrophic. In the intervening areas the tubules were well preserved. The blood vessels in the zones of fibrosis were moderately to markedly thick-walled and were surrounded by a dense zone of proliferated connective tissue.

The denervated kidney weighed 21 Gm. It presented essentially the same appearance as that of the kidney on which no operation was performed, except that the zones of fibrosis were neither as numerous nor as marked.

Dog 10—The left kidney was denervated on June 27, and the dog was killed on September 5.

The normal kidney weighed 35 Gm and grossly appeared normal.

Microscopically the glomeruli were well preserved, the tubular epithelium was moderately narrowed, and the free margins of the cells were frayed. A moderate amount of granular debris was present in the tubular lumens. In a few foci there was proliferation associated with round cell infiltration of the interstitial connective tissue.

The denervated kidney weighed 27 Gm. The capsule was thickened and adherent.

Microscopically the glomeruli were large, there was moderate atrophy of the loops, and the capillaries were distended with red cells. Bowman's space was free from contents and the basement membrane of Bowman's capsule was moderately proliferated. There was scattered round cell infiltration of the interstitial tissue and marked atrophy of the tubular epithelium. The arterioles were thick-walled and hyalinized, and their lumens were markedly diminished in caliber.

Summary of Group 3—In one of the two dogs which survived the combined injections of colon bacilli and pitressin, the denervated kidney showed approximately the same changes as those of the normal kidney whereas in the second dog marked fibrosis with interstitial inflammation was noted

COMMENT

In one animal of the earlier series of twenty-six dogs on which one of us reported¹ spontaneous diffuse atrophy and fibrosis of the denervated kidney were noted. In the present series of dogs which received injections of colon bacilli or of pitressin, or of both bacilli and pitressin, marked fibrosis associated with chronic inflammation in the denervated kidney developed in more than one half of the animals surviving for a month. These changes involved the interstitial tissue, the basement membrane of Bowman's capsule and, more markedly, the arterioles. The chronic, low grade inflammation, whether ascending or hematogenous in origin, was aggravated by the vascular changes resulting from denervation. Such pathologic changes are seen to a slight degree in old dogs, but never to the degree observed in these experiments. It is apparent that denervation in itself predisposes to the pathologic changes described and that the changes are hastened by a variety of agents which are capable of causing mild arteriolar injury.

The results can best be interpreted in the light of the function of the renal nerves and of the experimental results that have been obtained after denervation.

Although experimental work has been reported supporting the theory of a secretory function, the mass of evidence favors vasomotor and sensory control as a dominant, if not the only, function of the renal nerves.³ The removal or destruction of the nervous pathway gives rise to functional changes which can directly or indirectly be ascribed to the loss of this vascular control. Chief among these immediate changes are increase in the volume and diminution in the specific gravity, but there is increase in the amount of the total solids of the urine. Apparently the secretory pressure of the urine remains unchanged.⁴

Rhoads and his associates⁵ were unable to demonstrate a consistent change in renal blood flow or urea clearance after denervation or

3 Gironcoli, F. *Ztschr f urol Chir* **27** 266, 1929. Hesse, E. *Surgery of the Vegetative Nervous System*, Moscow, Staatsverlag, 1930, p. 243. Kuntz, A. *The Autonomic Nervous System*, Philadelphia, Lea & Febiger, 1929, p. 271. Ellinger, P. A. and Hirt, A. *Arch f exper Path u Pharmacol* **106** 135, 1925.

4 Bieter, R. N. *Proc Soc Exper Biol & Med* **26** 792, 1929.

5 Rhoads, C. P., Van Slyke, D. D., Hiller, A., and Alving, A. S. *Am J Physiol* **110** 392, 1934.

cocainization of the renal pedicle of the transplanted kidney in dogs from which the opposite kidney had been removed. Their experimental methods were not entirely comparable to those of the other workers in the field. Their work indicates a marked automaticity of the renal vascular bed, which resulted in its control in the absence of the normal nerve supply and which probably accounts, to some extent at least, for the inconsistency in our results. This conclusion is supported by the fact that the vascular response of the denervated ear of the rabbit to experimental fever is similarly inconsistent (Pinkston⁶).

The denervated kidney presents a definitely altered response to toxic agents, such as corrosive mercuric chloride, uranium acetate, cantharides and snake venom, in that it appears to be more susceptible to damage than does the normal kidney. This may be explained by the inability of the denervated kidney to protect itself against toxic agents through vasoconstriction.

Of special importance are the reactions of the normal and the denervated kidney to bacteria which may be present in the blood stream. Hecht,⁷ using rabbits, demonstrated that bacterial emboli are fewer and abscesses less numerous in the denervated than in the normal kidney after a single intravenous injection of a suspension of staphylococci. Muller and his associates⁸ reported that with a continuous intravenous injection of a suspension of colon bacilli, after from forty to sixty minutes when chilling occurred, the bacteria as well as blood and albumin were excreted in the urine from the normal kidney. The blood cells and albumin give evidence of renal injury. The denervated kidney continued to excrete sterile and clear urine. He presumed that vasoconstriction occurring with the chill lowered the resistance of the renal tissue to the point at which the kidney was unable to withstand the injurious effects of the bacteria. In order to test this hypothesis further he first chilled the animals by means of an ice pack and then began continuous intravenous injections of colon bacilli. He reported that, instead of an interval of from forty to sixty minutes before changes were noted, bacteria, blood and albumin were found almost immediately in the urine from the normal kidney, whereas the denervated kidney continued to excrete urine which was apparently unchanged. One of us⁹ studied the urine collected separately from normal and denervated kidneys and observed certain phenomena in connection with the intravenous injection of bacteria. After a single intravenous injection of

6 Pinkston, J. O. *Am J Physiol* **110** 448, 1934.

7 Hecht, R. *Proc Soc Exper Biol & Med* **29** 212, 1931.

8 Muller, E. F., Petersen, W. F., and Rieder, W. *Verhandl d deutsch Gesellsch f inn Med* **42** 580, 1930.

9 Milles, G., and Nedzel, A. J. *Proc Soc Exper Biol & Med* **29** 976, 1932.

a suspension of *Bacillus prodigiosus* the urine from the normal kidney contained the organisms in far greater numbers than did that from the denervated kidney. Previous injury with snake venom decreases the ability of the normal kidney to excrete bacteria but does not alter the relative rates of bacterial excretion of the normal and denervated organs.

The work of Dastre and Morat,¹⁰ amply corroborated and extended by Petersen and Muller,¹¹ demonstrated a balance of vasomotor tonus between the peripheral, or better the extraperitoneal and the intra-peritoneal splanchnic vascular bed. Briefly, this consists in the two divisions of the vascular bed of the organism being in opposite states of vascular tonus. In this grouping the kidneys are oriented with the skin. In experiments with acute injury it is apparent that the vasomotor nerves of the kidney cause constriction in the renal vascular bed during a chill concomitant with vasoconstriction in the skin. With the vasoconstriction in the kidney the mechanical effect of washing with a large volume of blood which normally interferes with the localization of bacteria (stoppage) is lost. More important, however, is the diminution of the blood supply to actively metabolizing tissues, with the resultant local anoxemia and failure of removal of tissue metabolites. These factors lower the local resistance of the tissues and markedly increase the tendency of bacteria to localize. The normal fluctuations in the renal vascular bed which produce areas of vasoconstriction and vasodilatation have a similar, but much less marked effect. Exposure and chilling, such as are a common experience of persons in the rigorous climates, have perhaps even more effect in causing the localization of bacteria in the kidneys. Thus a common history of onset in acute nephritis is one in which the complaint began with exposure and chilling, which, as we have experimental evidence to prove, produce marked peripheral and renal vasoconstriction. Conversely, with the surgical removal of the renal nerve supply a more or less permanent vasodilatation occurs, which is unaffected by the nervous orientation of the rest of the organism. In experiments dealing with acute infection it prevents the excretion of bacteria into the urine and defeats the localization of bacteria with the resultant injury to the tissue of the kidney by the organisms.

Destruction of the renal nerve supply appears to have an entirely different effect if the animal is subject to repeated insults judging from the experiments which we have already detailed. In analyzing these results the fact that the denervated kidney is likely to be damaged

10 Dastre, A., and Morat, J. P. *Recherches experimentales sur le systeme nerveux vaso-moteur*, Paris, G. Masson, 1884.

11 Petersen, W. F., and Muller, E. F. *Arch. Int. Med.* **40** 575, 1927.

severely by daily intravenous injection of pitressin or colon bacilli, or both, is apparent. In order to explain this one must conclude that when deprived of the normal nerve supply the renal vascular bed goes into a state of atonic dilatation and that this results, after a period of time, not in improved but in reduced renal circulation as the result of stasis. Under these circumstances the denervated kidney would be protected against the spastic effects of a single injection of a noxious agent. When, however, the insult is repeated daily for a period of time direct injury to the tissues occurs, as is indicated by a low grade proliferative inflammation, the result of the summation effects of the circulatory disturbance and the noxious agent. Theoretically, the same effects might be observed after denervation even without the injection of an injurious agent since the degree of dilatation and stasis obtained must vary in different animals. This was noted but once in a series of twenty-six dogs.¹² Physiologically, renal function is usually unimpaired for as long as two months after bilateral denervation without the injection of a noxious agent (Caldwell, Marx and Rowntree¹³). Nisio¹⁴ and Seres¹⁴ observed degenerative changes in the kidney after denervation in some animals. The kidneys, which are observed to be the site of the marked degenerative changes described, are pale on gross examination, owing to anemia which is the result of the marked vascular fibrosis seen histologically.

In short, after denervation the renal vascular bed undergoes a period of dilatation and improved circulation lasting but a few weeks or months. This gives way to a period of dilatation and stasis. The resulting stasis and anoxemia plus an additional noxious factor, or occasionally the first condition without the second agent, gives rise to a low grade interstitial inflammation with marked proliferation of connective tissue.

Much the same condition may be visualized as occurring even in the normal kidney. As the result of the summation of the normal wavelike fluctuations in the vascular tone fortified by marked fluctuations in the environmental factors, especially the meteorological,¹⁵ the kidney may be in a state of dilatation and stasis, equivalent to denervation, for variable periods. Under these circumstances, transient bacteremia or toxemia, encountering the kidney in a state of minimal resistance would inflict damage mounting with each recurrence.

12 Caldwell, J. M., Marx, H., and Rowntree, L. G. *J. Urol.* **25** 351, 1931.

13 Nisio, cited by Gironcoli.³

14 Seres, M. *Rev. med. de Barcelona* **1** 220, 1924, abstr., *Ztschr. f. urol. Chir.* **17** 54, 1925.

15 Petersen, W. F., and Milliken, Margaret E. *The Patient and the Weather*, vol. III *Mental and Nervous Diseases*. Vol. II *Autonomic Dysintegration*, Ann Arbor, Mich., Edwards Brothers, Inc., 1934.

SUMMARY

After denervation the dog's kidney was observed to be subject to mild chronic inflammatory and marked degenerative changes when it was subjected to repeated injections of colon bacilli or pitressin, or of both. We conclude that this is the direct result of the loss of nervous control with attendant dilatation and stasis lowering the resistance of the tissue to the injurious agents to which the organism was subjected repeatedly for long periods of time.

MECHANISM OF ACUTE INFLAMMATION

VIRGIL H. MOON, M.D.

PHILADELPHIA

This discussion will be limited to the early vascular and cellular phenomena which develop in and about an area of local injury to the tissue. This response to an injury consists essentially in changes in the caliber and permeability of vessels, the development of an exudate locally and leukocytosis both general and local. The subsequent phases of local inflammation will not be discussed. A group of observations furnished by various workers may be assembled and correlated into an intelligible picture of the mechanism of inflammation.

Ebbecke's¹ physiologic studies on vascular reactions to irritation suggested a new interpretation of such reactions. His results illustrated Pflüger's dictum that physiologically the conditions giving rise to a need likewise provide for supplying it (*Die Ursache des Bedürfnisses zugleich die Ursache für die Befriedigung des Bedürfnisses*). He showed that irritational and inflammatory hyperemia result not from nerve stimuli but from substances released locally by tissue cells and that following mechanical, electrical or chemical stimulation such substances affect the adjacent vascular structures, causing dilatation, slowing of the blood flow, increased permeability of the capillaries and edema. This capillary-dilating substance was similar in its action to Heidenhain's lymphagogues and to the substances which produce urticaria, foreign protein reactions and the like. Ebbecke explained the cellular proliferation seen in later stages of inflammation as being due to a "wound hormone" derived from the injured tissue. This interpretation is supported by the recent demonstration of growth-stimulating substances such as trephones both *in vivo* and *in vitro*.

The mechanism of local vascular reactions in human skin was studied extensively by Lewis² and his associates. Mechanical or other forms of irritation are followed by a reaction with uniform characteristics regardless of the type of irritation. This reaction consists in (1) local dilatation and hyperemia of the minute vessels, (2) a spreading flare resulting from reflex dilatation of adjacent arterioles and (3) a pale circumscribed wheal resulting from increased permeability of the walls

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1 Ebbecke, U. *Arch. f. d. ges. Physiol.* **169** 1, 1917, **199** 197, 1923

2 Lewis, Thomas. *Blood Vessels of the Human Skin and Their Responses*, London, Shaw & Sons, 1927

of the capillaries. Chemical examination of the fluid from such wheals revealed it to have a protein content closely approximating that of blood plasma. It had a higher protein content than that of fluid from the lymph spaces of the same limb or than that of edema fluid from dropsical patients.

The complete triple response occurred in areas in which the sensory innervation had been interrupted recently. However, in skin anesthetized by nerve block with cocaine the local red reaction and the wheal occurred as in normal skin, but the spreading flare was absent. Neither did the flare occur in areas where complete degeneration of cutaneous nerves had occurred. For these reasons the flare was explained as an axon reflex resulting in dilatation of adjacent arterioles, but the local dilatation of capillaries and the edema were caused by some mechanism independent of innervation.

The triple response was produced characteristically by various agents. These included histamine, burning, freezing, faradic current, intense light, various acids, alkalis and salts, mustard oil, cantharidin, nettle stings, bites and stings of insects, peptone and foreign proteins. Bacterial toxins produced similar results but with variations in the time factor.

Lewis presented an impressive array of evidence derived from varied experiments which supports the conclusion that this reaction is produced not by the irritant itself but by a substance released from the cells in response to the irritation. The injection of watery extracts of normal human epidermis into the skin produced wheals indistinguishable from those produced by solution of histamine. Numerous analogies were found between this substance and histamine. He concluded that the mechanical stroke or any other form of irritation to the skin produces injury to the deep epidermal cells and that in response to this injury the cells liberate a diffusible substance which acts like histamine on the walls of the capillaries, causing them to dilate and to become more permeable. Lewis could not distinguish this substance from histamine, but lacking absolute chemical identification he spoke of it as H-substance. He stated that whenever the skin displays an acute reaction in the form of the triple response that reaction is provoked by H-substance.

Harris³ found that histamine is released in a diffusible form following burns made on the skin of mammals. He made physiologic assays for histamine in normal human epidermis and found from 22 to 24 mg of histamine per kilogram. Kalk⁴ and Harmer and Harris⁵ corroborated this finding. Histamine has been demonstrated by many

³ Harris, K. E. *Heart* **14** 161, 1927.

⁴ Kalk, H. *Ztschr. f. klin. Med.* **109** 118, 1928, *Klin. Wchnschr.* **8** 64, 1929.

⁵ Harmer, I. M., and Harris, K. E. *Heart* **13** 381, 1926.

workers (Best and McHenry⁶) in similar amounts in tissue of the lung and gastro-intestinal mucosa and in varying amounts in other tissues. Loos⁷ produced acute serous inflammation in rabbits' ears by the application of hot water. He extracted histamine from the inflamed ear in eight times the concentration found in the normal ear of the same rabbit. He found also that histamine in a dilution of 1:40,000 increased the phagocytic activity of leukocytes *in vitro*. Other reports have suggested a relationship between histamine and phenomena associated with injury to tissue.

Dale⁸ reviewed and corroborated Lewis' investigations and conclusions and stated

If H-substance is not histamine it is a substance which immediately yields it when the simplest and least injurious procedures which we can devise are applied to its extraction from the cells and its isolation for chemical study. We have as good chemical evidence of the presence of histamine in the cells of the body generally, and of its liberation from them under certain conditions, as we have of the existence of adrenalin in the suprarenal medulla, and of its secretion as such into the blood.

Krogh⁹ endorsed the conclusion that histamine or H-substance is liberated from the skin of mammals whenever and however the cells are injured and that it affects the capillaries in two particulars. It causes them to dilate, producing local hyperemia, and it increases their permeability, causing local edema and swelling.

Lewis found that both the triple response to any injury and the reaction to a solution of histamine pricked into the skin produced a local increase in temperature. Kling¹⁰ found that the therapeutic application of histamine to the skin by cataphoresis increased the local temperature by 2 or 3 C. Histamine even in high dilution is irritating to sensory nerves and produces pain. This factor plus the increased tension resulting from edema may account for the pain which develops following local injury to cells. The local cutaneous reactions to histamine result in heat, redness, pain and swelling, the cardinal signs of acute inflammation. It is self-evident that three of these are merely the visible evidences of vascular changes.

The observations cited may be summarized as follows. The cells of normal tissues contain some combination of histamine in a nondiffusible form. The amounts of this are greater in tissues which are

6 Best, C. H., and McHenry, E. W. *Physiol. Rev.* **11**: 371, 1931.

7 Loos, H. O. *Arch. f. Dermat. u. Syph.* **164**: 199, 1931, *Ztschr. f. d. ges. exper. Med.* **75**: 463, 1931.

8 Dale, H. H. *Lancet* **1**: 1235, 1929.

9 Krogh, A. *Anatomy and Physiology of Capillaries*, ed. 2, New Haven, Conn., Yale University Press, 1928.

10 Kling, D. H. *Ann. Surg.* **99**: 568, 1934.

most subject to injuries, such as the skin, the respiratory system and the gastro-intestinal tract. The cells when injured liberate histamine locally in a diffusible form. It affects the adjacent capillaries, causing them to dilate and to become unresponsive to substances or nerve impulses which produce contraction. Also, it increases the permeability of the capillary endothelium, resulting in transudation of plasma which produces edema. If the sensory nerve fibers are intact histamine causes reflex dilatation of neighboring arterioles, thus increasing the flow of blood. The vascular phenomena of inflammation, resulting in dilatation of arteries and capillaries, congestion, edema and swelling, have received an acceptable explanation.

These observations raise the question of what relationship may exist between histamine and the leukocytic phenomena associated with inflammation. This question may best be discussed under general (blood stream) and local leukocytosis. Few studies have been made on the effects of histamine on the leukocytes in the blood. Lieber, Kennedy and I¹¹ have reviewed the literature and have reported on the effects of histamine administered intravenously and subcutaneously to cats, monkeys and human subjects. Single intravenous injections of from 1 to 2 mg. were followed regularly by a marked increase in the number of polymorphonuclear cells in the blood of cats. The number returned to normal within twenty-four hours. Subcutaneous injections of histamine into monkeys were followed regularly by marked leukocytosis. The effects appeared to vary with the dosage and the count remained above normal for three or four days. Following the injection of 0.75 mg. the count rose from 5,400 to 18,850 within two hours. Following the injection of 9 mg. the count rose from 5,550 to 57,550 within one hour. In human subjects the effects were much less marked, perhaps because of the small dosage in proportion to the body weight. The intravenous injection of from 0.5 to 1 mg. was followed by a slight increase in the number of polymorphonuclear cells in the blood. Subcutaneous injections of from 2 to 3 mg. produced no significant changes. Injections of 5 mg. produced a moderate increase in the number of polymorphonuclear cells in the blood.

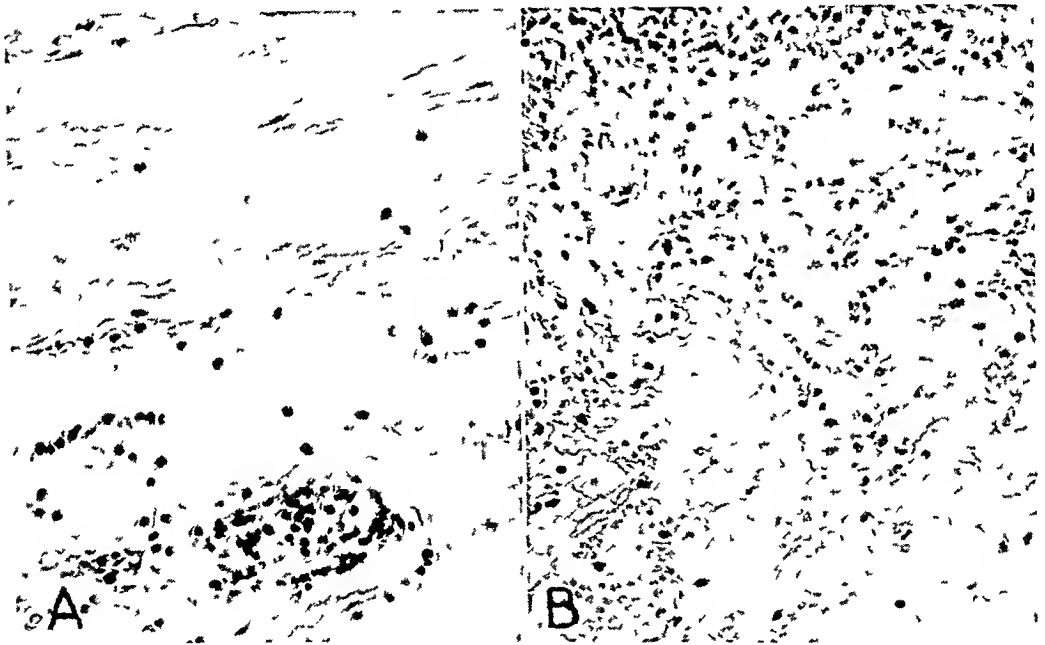
We concluded that the injection of histamine phosphate causes leukocytosis. However, there is a question whether the degree of this relative to the amount injected is proportionate to the leukocytosis associated with extensive inflammation. Such leukocytosis may be due in part to histamine and in part to some other factor associated with inflammation. Or the form in which histamine exists in the tissues may be more effective than is histamine phosphate.

¹¹ Moon, V. H., Lieber, M. M., and Kennedy, P. J. *Arch. Path.* **20**: 209, 1935.

Attention is now directed toward the local accumulation of leukocytes in and about areas of injury to the tissues. A mild local burn furnishes conditions suitable for observations on this phenomenon. It is a simple injury uncomplicated by the presence of bacteria or of any foreign substance. No external agent or factor is introduced. The reaction to such an injury must depend entirely on endogenous processes.

EXPERIMENTAL OBSERVATIONS

Experimental burns were made on guinea-pigs. A metal rod 5 mm in diameter with smooth ends was sterilized and heated in water at 90 C. The end of this rod was held against the skin of the abdomen of a guinea-pig for ten seconds.



Photomicrographs of (A) subcutaneous tissue one hour after a burn. Marked edema is present and the migration of leukocytes is beginning. B shows subcutaneous tissue eight hours after a burn. Inflammatory edema, migration of leukocytes and diapedesis of red cells are marked. The accumulation of leukocytes in the upper part of the field is just beneath the burned area.

The entire abdomen had been clipped, shaved and sterilized as for a surgical operation. The possibility of infection was minimized by covering the abdomen with a sterile gauze dressing following the burn. A series of such burns was made at intervals on the same guinea-pig, after which sections of the skin were prepared for microscopic study. Within one hour the capillaries and venules were widely dilated in the deep cutaneous and subcutaneous tissues, distinct edema was present, and the migration of leukocytes was beginning (figure). At later periods the leukocytes were progressively more numerous and had wandered away from the vessels. This migration was not uniform in all directions but was from the vessels and toward the burned areas. The evidence indicated that some substance was attracting the leukocytes into the areas of injury. Repetitions of this experi-

ment gave uniformly similar results. Those who have not made a study of acute inflammation, the time factor of which is known definitely, have an imperfect idea of the speed with which the reaction develops in previously normal tissue. The vascular response is immediate, the development of edema begins within a few minutes, and leukocytic migration begins within an hour. Within eight hours all the features of acute inflammation are fully developed. The area is hyperemic and swollen, the tissue fibers are separated by marked edema and a marked leukocytic infiltration is present (figure, *B*).

In another experiment blisters were raised by burning human skin in areas previously sterilized as for a surgical procedure. The mouth of a test tube 5 mm in diameter filled with water at 80 C was held against the skin for five seconds. A characteristic hyperemic reaction with surrounding flare developed immediately. Shortly the edematous swelling caused separation of the superficial epithelial layer, and a bleb was formed. Counts of the leukocytes in such blister fluid were made at varying intervals following such burns. A few representative counts on such fluid, together with the elapsed time following the burns, are given in the following tabulation.

Leukocytes	Elapsed Time, Hours
2,750	3
4,700	3
8,600	3
2,400	6
12,650	6
17,350	6
21,200	24
27,650	24

This migration of leukocytes into blister fluid apparently was not due to bacteria. The areas were sterile, and no bacteria were found in the fluid. Neither was it due to other extraneous agents for no foreign substance of any kind was introduced. One cannot escape the conclusion that the substance which attracted leukocytes was derived from injured cells.

Others have made observations on leukocytosis following burns. Pack¹² reported that what he called "burn toxin" was chemotactic for leukocytes. Schattenberg and Harris¹³ demonstrated that local leukocytosis resulted when rabbits' ears were immersed in water at a temperature of 44 C. The untreated ears of the same rabbits were used as controls. Pack, Underhill,¹⁴ Askanazy¹⁵ and others have attested the fact that generalized leukocytosis follows extensive superficial burns.

12 Pack, George T. Arch Path **1** 767, 1926

13 Schattenberg, H. J., and Harris, W. H. Proc Soc Exper Biol & Med **29** 269, 1931, **29** 1052, 1932

14 Underhill, F. P., Kapsinow, R., and Fisk, M. E. Am J Physiol **95** 315, 1930

15 Askanazy, M., in Aschoff, L. Pathologische Anatomie, ed 7, Jena, Gustav Fischer 1928, vol 1, p 69

Locke¹⁶ made studies of the blood immediately following such burns in ten patients. Marked leukocytosis was present in every case. The counts ranged between 10,000 and 50,000 within two and one-half hours. He found that marked leukocytosis appeared within an hour and that it was progressive and proportionate to the severity of the burn. Regularly in fatal cases the count was above 50,000 before death. I know of no other condition capable of producing so high a leukocyte count in so short a time. It is as if a sudden call to arms had roused myriads of defenders from their tents. The question is whether the alarm was telegraphed by way of nerve fibers or was carried to the bone marrow and other distant parts by the subtle means of a substance released by injured cells.

The evidence thus far indicates that systemic leukocytosis follows the injection of histamine in experimental animals and in man, that systemic leukocytosis follows extensive superficial burns and that leukocytes are attracted toward burned areas by some substance released locally as a result of the injury. The question is whether that substance is histamine.

Wolf¹⁷ reported that a 0.000025 per cent solution of histamine was strongly chemotactic. However, Bloom,¹⁸ Paul¹⁹ and others were unable to find evidence that histamine has any chemotactic effect. Following the technic which Wolf described and with modifications of it *in vitro* I did not note increased migrations of leukocytes toward inert substances containing varying concentrations of histamine. Experiments were then devised to secure further evidence on this point *in vivo*.

In one series of experiments cylindric bits of elder pith of uniform size, 4 by 10 mm., were first soaked in ether, in alcohol and in salt solution for twenty-four hours each to remove any soluble substances. These were then saturated with physiologic solution of sodium chloride containing histamine phosphate in a 1:10,000 solution and were implanted in animal tissues through a canula by the method devised by Konzelmann and me²⁰. Some were introduced into the peritoneal cavity and others into the loose subcutaneous areolar tissue. In every instance a bit of elder pith saturated with physiologic solution of sodium chloride was similarly implanted as a control. Plugs saturated with histamine were implanted in nine monkeys, eight cats and four guinea-pigs. After from six hours to twenty-four hours the animals were killed and the reaction about the histamine pith was compared with that of the control. In every instance there was a marked zone of congestion about 2 cm. in diameter surrounding the pith saturated with histamine. There was none about the controls. The capillaries and venules in the former areas were markedly distended, congested and prominent as compared with those around the controls. Microscopic examination revealed numer-

16 Locke, E. A. *Boston M. & S. J.* **147** 480, 1902.

17 Wolf, E. P. *J. Exper. Med.* **35** 375, 1921, **37** 511, 1923.

18 Bloom, W. *Bull. Johns Hopkins Hosp.* **33** 185, 1922.

19 Paul, J. R. *Bull. Johns Hopkins Hosp.* **32** 20, 1921.

20 Moon, V. H., and Konzelmann, F. W. *Arch. Path.* **10** 587, 1930.

ous leukocytes about both test and control piths. There was no evidence of active invasion of the pith by cells, and leukocytes were apparently as numerous about the control piths as about the ones containing histamine.

In another series of animals histamine was mixed with neutral 2 per cent agar and was injected subcutaneously. Injections of agar without histamine were used as controls. Congestion was more marked about the histamine and agar, but leukocytosis was equally marked about each.

These experiments *in vivo* indicated that histamine produces marked hyperemia, especially of the capillaries and venules, when introduced into living tissue. There was complete absence of evidence that histamine phosphate attracts leukocytes locally. In each of the implantation experiments some degree of local injury to the tissue was produced. The evidence indicates that some product of cellular injury other than histamine is responsible for the local attraction of leukocytes. These interpretations are in agreement with the conclusions of Morgan²¹ from similar experiments that the chemotactic substance is not histamine but is some other product of injury to the tissue.

COMMENT

A wealth of literature has resulted from efforts to discover what functions histamine serves in the physiology of normal tissues. There is agreement that histamine in very low concentration causes capillaries and venules to dilate and to become more permeable. It appears that the immediate vascular reaction to local injury to the tissue—the vascular phenomena of beginning inflammation—results from this action. The experiments by Lieber and me indicated that large amounts of histamine such as might be released from extensive injuries may cause a systemic mobilization of leukocytes. It appears that histamine produces other systemic phenomena associated with inflammation. Weiss, Robb and Ellis²² made studies on various manifestations following slow continuous injection of histamine into human subjects. The quantities given were not sufficient to produce such circulatory disturbances as follow the sudden administration of histamine in large amounts. They noted an increase in the cardiac rate, the cardiac output was increased 20 per cent, and the basal metabolic rate was increased from 15 to 50 per cent. Concurrent observations on body temperature would be of interest had they been recorded.

My experiments indicated that the substance which attracts leukocytes into areas of injury to the tissue is derived from injured cells. They also indicated that this attraction is due to some other substance than histamine. Krogh suggested that other products of cellular dis-

21 Morgan, J. R. E. *Arch. Path.* **18** 516, 1934.

22 Weiss, S., Robb, G. P. and Ellis, L. B. *Arch. Int. Med.* **49** 360, 1932.

tegration are chemotactic. Lewis admitted that there may be more than one H-substance, some of which may have other properties than those of histamine. It would not be strange if the same substance which attracts leukocytes locally should also be effective in producing systemic leukocytosis.

The deposition of fibrin in areas of inflammation was not made the subject of study. It was shown by Lewis that the edema fluid in wheals is similar in composition to blood plasma. Such fluid contains fibrinogen and other factors necessary to the formation of fibrin in the presence of injured cells. Apparently the presence or absence of fibrin in inflammation is conditioned on the degree of increased capillary permeability. If this is increased only slightly the edema contains less plasma proteins, and fibrinogen may not be present in it.

All the phenomena of acute inflammation are explainable as local reactions to substances released by injured cells. In the language of Lewis, the agent that alarms the garrison and mobilizes the vascular defenses is a chemical agent derived from the tissues. The perfection of this mechanism is such that the defense is organized immediately and at every threatened point, it is arranged and carried through locally, being independent of higher systems of control (nervous) and of distribution (cardiovascular). It develops and runs its characteristic course even in denervated areas.

Complex animal organisms are endowed with several physiologic mechanisms which are operative only in emergencies. These are protective and are automatically activated by some factor inherent in the emergency itself. Examples of such mechanisms may be seen in the increased discharge of epinephrine into the blood in response to pain, fear or other emotions, the coagulation of blood following injuries to the walls of the vessels, and the development of immunity as a reaction to certain infections. The inflammatory reaction may best be interpreted as belonging in this group of physiologic mechanisms. It is the local reaction of vessels and cells (leukocytes) to an injury. It is protective in character and purpose, designed to lessen the effects of the injury and to facilitate repair and restitution.

A mechanism by which injured tissues may themselves initiate a local and systemic defensive reaction independent of nerve impulses and of remote control is admirably adapted to the purposes of defense and repair.

CONCLUSIONS

The local vascular and cellular phenomena of acute inflammation result from the liberation of substances from injured cells.

One such substance which apparently is some combination of histamine produces the vascular reactions resulting in congestion,

capillary dilatation and permeability, edema and local elevation of temperature

Systemic leukocytosis results from the injection of histamine phosphate into animals. Histamine released from extensive areas of injured tissue is probably a factor in producing the resulting leukocytosis.

A substance released from injured cells attracts leukocytes to the area of injury. This same substance may also be effective in systemic leukocytosis. Apparently this substance is not histamine.

There is evidence that increase in the metabolic rate and in the rapidity of circulation follows the injection of histamine. This may be a factor in the systemic reactions which accompany extensive inflammation.

LATE CHANGES IN THE LIVER INDUCED BY MECHANICAL OBSTRUCTION OF THE HEPATIC VEINS

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AND
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Numerous methods have been employed in attempts to produce experimental cirrhosis of the liver. As shown in the recent review of the subject by Moon,¹ most investigators in this field have used some poison which, in most instances, caused varying degrees of fibrosis rather than typical cirrhosis. Permanent occlusion of the hepatic veins by obliterating endophlebitis, reviewed by Hess² in 1905, by Meyer³ in 1918 and by Satke⁴ in 1929, induces varying degrees of fibrosis of the liver. No one appears to have studied the late effects of temporary occlusion of the hepatic veins, although Simonds and Callaway⁵ have reported the changes in the livers of dogs during the first seven days following such operative procedure. This paper is a report of the anatomic alterations and the functional changes in the livers of twelve dogs following mechanical obstruction of the hepatic veins by the method described by Simonds and Brandes⁶ for periods of from ten to fifty minutes. The animals were put to death at intervals from the seventh to the sixtieth postoperative day.

OBSERVATIONS

The dogs were selected for these experiments without regard to age or sex. Pentobarbital sodium given intravenously in doses of from 30 to 35 mg per kilogram of body weight was used as an anesthetic in all experiments except the last two, in which morphine, atropine and ether were employed. The mechanical occlusion of the hepatic veins was maintained for thirty minutes in all except three dogs. In these three animals the periods of occlusion were ten, eighteen and fifty minutes, respectively. During obstruction of the hepatic veins the liver became

From the Department of Pathology, Northwestern University Medical School

1 Moon, V H Arch Path **18** 381, 1934

2 Hess, A F Am J M Sc **130** 986, 1905

3 Meyer, O Virchows Arch f path Anat **225** 213 1918

4 Satke, O Deutsches Arch f klin Med **165** 330, 1929

5 Simonds, J P, and Callaway, J W Am J Path **8** 159, 1932

6 Simonds, J P, and Brandes, W W Am J Physiol **72** 320, 1925

greatly enlarged, cyanotic, tense and firm. Immediately following release of the veins it rapidly decreased in size and again had the appearance and feel of a normal liver. As soon as the dogs recovered from the immediate effects of the operation all except dog 10 ate heartily and appeared to be in good health until put to death.

Hepatic Function—Bromsulphalein tests of hepatic function were made on all animals. From 2 to 4 mg. of the dye per kilogram of body weight was given intravenously, and blood specimens treated with oxalate were collected fifteen and thirty minutes after administration. We have considered a 15 per cent retention of bromsulphalein in the plasma at fifteen minutes when a 4 mg. dose was employed to be the maximal normal retention in the dog. In only one instance (dog 15) did we observe a significant retention following operation. Twenty-four hours after operation, with a dose of 4 mg. per kilogram of body weight, there was from 50 to 60 per cent retention of bromsulphalein at fifteen minutes and 20 per cent retention at thirty minutes. Seventy-two hours after operation, 20 per cent of the dye had been retained at fifteen minutes and a mere trace at thirty minutes. The lipase test (see next paragraph) was negative in this dog. The pathologic changes in the liver when the dog was killed with chloroform on the thirty-fifth post-operative day were no more marked than in other animals in group 1 described later.

Blood Lipase—The blood lipase was investigated in eight dogs. The technic employed differed from that suggested by Cherry and Crandall⁷ only in that a control test was made with each determination. These authors stated that both in dogs and in man lipase capable of splitting olive oil appeared in increased amounts in the blood only when pathologic changes were present in the liver and pancreas. Our results have been expressed in the number of cubic centimeters of twentieth-normal sodium hydroxide required for neutralization of the fatty acid liberated by the lipase. A reading of 0.2 cc. of twentieth-normal sodium hydroxide has been considered the maximum of experimental error and the maximum normal lipase content of the blood. No significant rise was found in three dogs (6, 14 and 15), while in five there was a distinct rise following operation (table). The titer of the lowest maximal increase of blood lipase in these five dogs was 0.8 cc. of twentieth-normal sodium hydroxide in dog 8, the highest was 6.65 cc. in dog 13. The highest titers of blood lipase were noted during the first five days following operation. In four dogs the blood lipase returned to normal levels by the tenth postoperative day while in one animal (13) it was not investigated after the fourteenth postoperative day, although at that time it had not returned to normal. If the present conception of the significance

⁷ Cherry, I. S., and Crandall, L. A. *Am J Physiol* **100**: 266, 1932.

of this enzyme that splits olive oil is correct, these five animals should be considered as having had either preoperative or postoperative pancreatic or hepatic damage. Three of these dogs (8, 10 and 11) were killed on the seventh, fourteenth and ninth postoperative days, respectively; and belong in group 1, the other two (9 and 13), each killed on the sixtieth day after operation, were in group 2. Inasmuch as the increase in blood lipase occurred in the first ten days after occlusion of the hepatic veins, it is possible that the changes in the liver cells characteristic of group 1 may be associated with the increase of lipase in the blood. This supposition is, in a measure, in harmony with the observations of Whipple.⁸ The three dogs with normal blood lipase belong in group 2.

The presence of an olive oil-splitting enzyme was demonstrated in the blood of dog 10 before operation. Whether the yellowish areas of fatty change in the liver observed at operation and present as "fatty infarcts" (Cesaris Demel,⁹ Marias¹⁰) in sections had any bearing on

*Results of Study of Blood Lipase in Dogs with Experimental Obstruction of
Hepatic Veins*

Cubic Centimeters of Twentieth Normal Sodium Hydroxide Required to Neutralize the Acid Liberated Lipase															
Dog	Days Before Operation		Days After Operation												
	2	1	1	2	3	4	5	6	7	8	9	10	11	12	13
8		0 0	0 80	0 40	0 00	0 10	0 00								
9		0 1	1 15	1 25	0 50	0 35	0 60	0 30	0 30	0 60	0 10	0 00	0 05	0 00	
10	0 50	1 30	0 80	0 50	2 40	1 75	1 40	0 60	0 80	0 65	0 80	0 80			
11		0 00	2 80	2 15	2 25	0 40	0 30	0 20							
13	0 0	0 0	0 30	5 80	6 65	6 10	3 40	0 90	1 40	1 10	1 20	1 90	1 20	0 90	0 60

the preoperative positive titer is uncertain, for we have noted the presence of lipase in the blood of a few supposedly normal dogs examined at random. This dog was seized with uncontrollable generalized convulsions and was put to death fourteen days after operation. These convulsions could not be adequately explained. The blood showed 95 mg. of sugar per hundred cubic centimeters. Unfortunately the blood calcium was not determined. The parathyroids, however, were slightly enlarged, compact and free from intercellular fat. It is probable, therefore, that the convulsions were not related to tetany. The changes in the liver were those described in group 1 in a subsequent paragraph and were apparently no greater than those in other dogs in this group which did not have convulsions. The pancreas and kidneys were essen-

⁸ Whipple, G. H. *Bull. Johns Hopkins Hosp.* **24**: 357, 1913.

⁹ Cesaris Demel, A. *Pathologica* **24**: 332, 1932.

¹⁰ Marras, S. *Pathologica* **25**: 798, 1933.

tially normal. Examination of the brain for Negri bodies and other changes revealed nothing that explained the convulsions. They may, therefore, have been associated with hepatic insufficiency.

Hepatic Changes—These dogs can be divided into two equal groups of six animals each on the basis of the type of changes in the hepatic cells.

1. In group 1 the hepatic cells were swollen and granular. In only two dogs (10 and 12) did sections stained with sudan III show appreciable amounts of fat. The nuclei were either normal in appearance or were pale or pyknotic. Occasionally one or more cells were without nuclei. The sinusoids were, in general, narrowed but distinctly visible. In all livers in this group there were scattered, irregular, usually small areas in which the sinusoids of one or more lobules were distended with blood. This type of change was more common in the earlier stages, although it was present in a dog killed on the fifty-second postoperative day.

2. In group 2 the hepatic cells were enormously swollen, their outlines very distinct, their cytoplasm clear and foamy, and their nuclei usually small and hyperchromatic or, sometimes, absent (fig. 1). This is believed to be hydropic degeneration or intracellular edema. Fat was present in the liver cells in dog 13 only, the animal which had the highest titer of lipase. The sinusoids were collapsed and distinguished only with difficulty. The lobules were practically bloodless except for an occasional isolated red blood cell squeezed in among hepatic cells. The sections had the appearance of a very fine mosaic, and the general histologic architecture was greatly obscured but still recognizable. This type of change was observed chiefly in the later stages—from the fourteenth to the sixtieth postoperative day.

The liver weight-body weight ratios varied from 2.23 per cent to 3.96 per cent, the average for the twelve dogs being 3.092 per cent. In five cases it was less than the average reported by Junkersdorf¹¹ (3 per cent) and Simonds and Brandes¹² (3.03 per cent) for normal dogs, while in seven cases it was greater than the normal average. The average ratio in group 1 was 2.82 per cent with 60 per cent below normal, in group 2 the average ratio was 3.24 per cent with 66 per cent above normal. No relationship existed between the length of time the veins were constricted and the ratio of the liver weight to the body weight. The highest ratio, 3.96 per cent, was in a dog put to death fourteen days after operation. The ratios in two dogs which survived sixty days were 3.22 per cent and 3.78 per cent, i. e., above normal. Simonds and Callaway⁵ found that in dogs killed from a few hours

¹¹ Junkersdorf, P. *Arch. f. d. ges. Physiol.* **200**: 443, 1932.

¹² Simonds, J. P., and Brandes, W. W. *Arch. Path.* **9**: 445, 1930.

to seven days after operation the liver weight-body weight ratios ranged from 3.57 per cent to 3.96 per cent with an average of 3.77 per cent.

Accumulations of cells similar to those described by Simonds and Callaway⁵ were noted in the sinusoids in all cases. Some of these cells contained an iron-bearing pigment. Occasionally a few liver cells adjacent to or within the larger accumulations were necrotic and thus these areas had the characteristics of the focal necrosis of typhoid fever described by Mallory.¹³ At sixty days these cell masses in the sinusoids were rarely seen, probably because, their mission of phagocytosis having been fulfilled, they disintegrated.

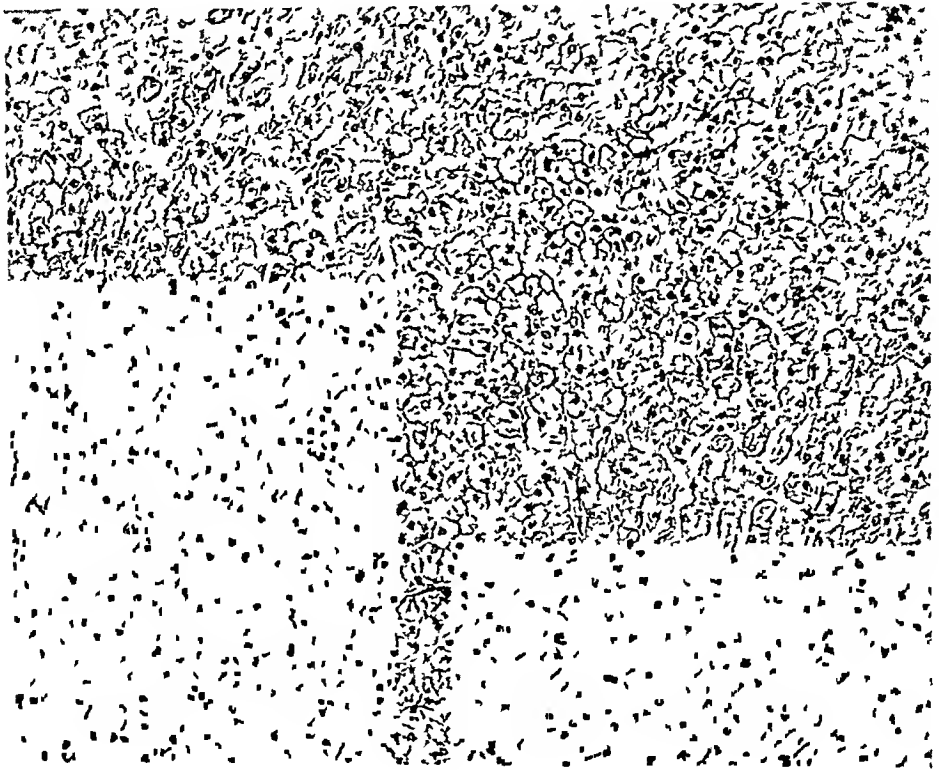


Fig. 1—Hydropic degeneration or intracellular edema in dog 9 on the sixtieth postoperative day, $\times 145$. Note the appearance of a fine mosaic, the distinctness of the cell outlines and the blurring of the normal histologic architecture.

A dilatation of the lymphatics around the sublobular, and sometimes about the portal, veins was noted in all twelve dogs, it was less prominent after the twentieth postoperative day. This phenomenon was also described by Simonds and Callaway⁵. In many instances the dilated lymphatics contained a pinkish-staining hyaline material. Similar homogeneous hyaline material was also present in the lumens of a varying number of central sublobular and portal veins.

13 Mallory, F. B. *J. Exper. Med.* 3: 611, 1898.

From the seventh to the twentieth day following operation accumulations of mononuclear cells were present around the central veins. The nuclei of most of these cells were large, round, oval or indented and stained well with hematoxylin. Among these large mononuclear cells were occasional lymphocytes and polymorphonuclear leukocytes. From about the twentieth postoperative day onward definite fibrous thickening of the walls of the central veins was evident. This became more marked as the postoperative period lengthened (fig 2 *A* and *B*). The connective tissue cells were frequently arranged radially about the stenosed central veins (fig 2 *A*). This was the only evidence of fibrosis in the livers of any of these dogs except for a slight increase of periportal connective tissue with moderate lymphocytic infiltration in dog 14 (group 1), killed on the fifty-second postoperative day. We have frequently observed an equal amount of periportal fibrosis in other dogs used in acute experiments.

Changes in Other Organs—The pathologic changes in other organs of these twelve dogs were relatively insignificant. Hyperemia of the spleen was present only in dog 3, in which the hepatic veins were constricted for only ten minutes and which was killed on the twentieth day after operation. In the other eleven dogs the spleen contained little blood, the pulp was moderately fibrotic, and hemosiderosis, not uncommon in old dogs, varied in amount. The pancreas was essentially normal in each animal in this series. The adrenals were normal except in dog 10, in which they were uniformly enlarged. The thyroid of this animal contained an adenoma, and its parathyroids were moderately enlarged, compact and free from intercellular fat.

The condition of the kidneys in these animals is important in view of the observations of Helwig and his co-workers¹⁴ who found serious renal changes in patients who had suffered severe, usually traumatic damage to the liver. In our dogs the glomeruli showed no noteworthy changes, although they varied in the amount of blood in the capillaries and the completeness with which they filled the capsular spaces. In a few dogs the epithelium of the proximal convoluted tubules was moderately swollen and granular, and the lumens contained varying amounts of granular material. In no case were casts present.

COMMENT

From these experiments it is evident that the dog's liver can withstand complete or almost complete stagnation of its circulation for periods of from thirty to fifty minutes, with relatively slight structural changes and functional disturbances. Using a method very different

14 Helwig, F. C. and Orr, T. S. *Arch Surg* **24** 136, 1932

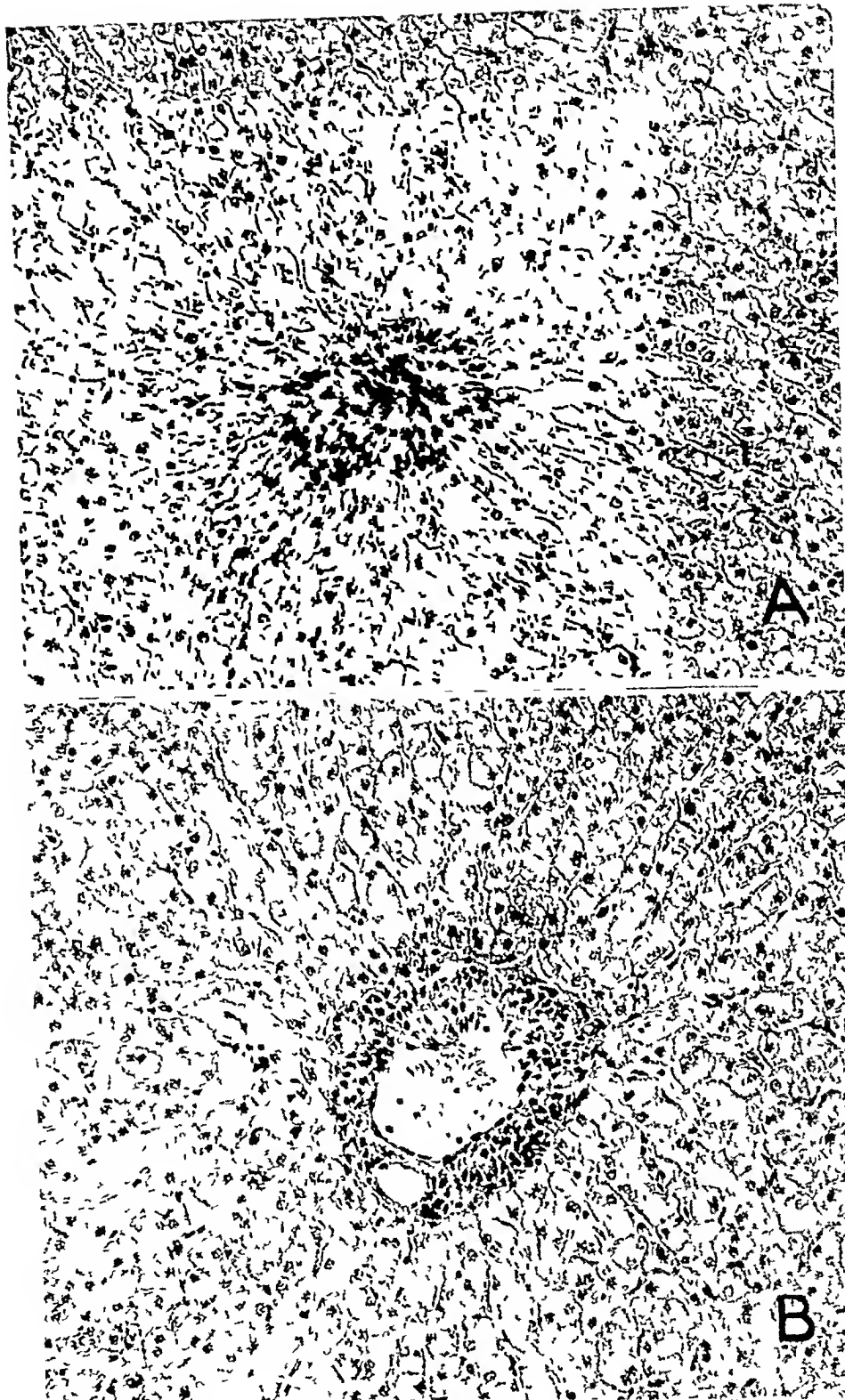


Fig 2—*A*, fibrosis about a central vein and radial arrangement of the connective tissue cells in dog 9, $\times 225$ *B*, fibrosis about a central vein in dog 13 on the sixtieth postoperative day, $\times 225$ Note the presence of channels in the fibrous tissue apparently for the passage of blood from the sinusoids to the vein *A* and *B* also show hydropic degeneration or edema of the hepatic cells

from ours, Chandler¹⁵ arrived at the same conclusion. This is in strong contrast to the effects of temporary stoppage of the circulation in other organs. Thus Gildea and Cobb¹⁶ found that ligation of the arteries of the brains of cats for ten minutes resulted in complete cessation of function of the ganglion cells with histologically demonstrable degeneration of these cells. Chandler¹⁵ reported that deprivation of oxygen for two hours causes almost complete necrosis of the epithelium of the renal cortex. McEney, Meyer and Ivy¹⁷ found that clamping of the renal vessels for from thirty to sixty minutes caused degenerative changes in the kidneys which often progressed to "the formation of a small white kidney" in dogs that survived for from ten days to seven months. The results varied with the extent of the collateral circulation.

This difference in resistance to anoxemia between the liver and other organs cannot be due to lack of specialization of the hepatic cells, for the liver possesses a greater number and variety of functions than any other organ of the body. Several factors may combine to produce this relative immunity of the liver to anoxemia. In the first place the hepatic cells are accustomed to a blood supply with a low oxygen content. Burton-Opitz¹⁸ has calculated that the total blood flow through the liver of the dog is 84 cc per minute for 100 Gm of liver. Of this, the portal vein furnishes 59 cc and the hepatic artery 25 cc. Of the total blood supply of the liver, approximately 70 per cent is venous and 30 per cent arterial. Normally, arterial blood contains 18.5 cc of oxygen per hundred cubic centimeters and is 95 per cent saturated, venous blood has 15 cc of oxygen and is about 70 per cent saturated (Wright¹⁹). The mixed blood in the hepatic sinusoids therefore contains only 16 cc of oxygen per hundred cubic centimeters and is only 78 per cent saturated. It is, thus, much more like venous than arterial blood. Cells which are accustomed to such a blood supply might well be expected to withstand stagnation of the circulation through the organ for a considerable time.

The liver normally uses relatively little oxygen. Winterstein²⁰ has presented evidence that the liver of the cat uses 1.1 cc of oxygen per hundred grams per minute. The kidney uses nearly 2.5 times, and

15 Chandler, L. R. *Proc. Soc. Exper. Biol. & Med.* **18** 24, 1920.

16 Gildea, E. F., and Cobb, S. *Arch. Neurol. & Psychiat.* **23** 876, 1930.

17 McEney, E. T., Meyer, J., and Ivy, A. C. *J. Lab. & Clin. Med.* **12** 349, 1927.

18 Burton-Opitz, R. *Quart. J. Physiol.* **4** 113, 1911.

19 Wright, Sampson. *Applied Physiology*, New York, Oxford University Press, 1926, p. 267.

20 Winterstein, H., in Bethe, A., von Bergmann, G., Embden, G., and Ellinger, A. *Handbuch der normalen und pathologischen Physiologie*, Berlin, Julius Springer, 1929, vol. 9, p. 541, cited by Weil, A. *Textbook of Neuropathology*, Philadelphia, Lea & Febiger, 1933, p. 76.

the brain about 9 times, that amount. Although the liver requires and uses little oxygen, it does not possess the power of anaerobic respiration with glycolysis and formation of lactic acid, as shown by Warburg²¹

Furthermore, during mechanical obstruction of the hepatic veins the outflow from the thoracic duct is increased 2.5 times (Simonds and Brandes²²). There is reason to believe that much of this increase is due to augmented filtration of fluid in the liver. This increased flow of lymph will mechanically remove some of the waste products that cannot be carried away by the blood while the outflow from the liver is obstructed.

Certain of the normal functions of the liver, such as detoxication, deamination, formation of urea and glycogenic activity, may be factors in protection of the hepatic cells against the effects of anoxemia.

Mechanical constriction of the hepatic veins for from thirty to fifty minutes produces three results that might well do permanent damage to any other organ, namely maximal distention of the sinusoids and veins, anoxemia and retention of the waste products of cell activity during the period of constriction. Six of our dogs showed evidence of temporary disturbance of hepatic function. In one animal there was retention of from 50 to 60 per cent of biomsulphalein at fifteen minutes, twenty-four hours after operation. In five, there was an increase of blood lipase lasting from two to thirteen days. One dog was killed on the fourteenth postoperative day because of uncontrollable generalized convulsions which, because they could not be explained on any other basis, were thought to be related in some way to hepatic insufficiency.

Two types of progressive, or at least continued, degenerative processes were observed. In one group the hepatic cells were swollen and granular as in ordinary parenchymatous degeneration. This change was still quite definite in two dogs killed thirty-five and fifty-two days, respectively, after operation. In the second group the liver cells were enormously swollen, and their cytoplasm was clear and foamy in appearance. This change was marked in three dogs, one of which was killed thirty-seven, the other two, sixty days, after operation. The hepatic cells were so swollen that the sinusoids were completely collapsed and, from the sections, the liver appeared to be almost bloodless. Ravdin²³ illustrates, without comment, such a change in the liver of a dog in which the common duct had been ligated.

The conditions observed by us in these dogs differed in several significant respects from those described by Simonds and Callaway⁵ from twenty-four to seventy-two hours and seven days after mechanical con-

²¹ Warburg, Otto, Posener, K., and Negelein, E. *Biochem Ztschr* **152** 309, 1924.

²² Simonds, J. P., and Brandes, W. W. *J Immunol* **13** 11, 1927.

²³ Ravdin, I. S. *J A M A* **93** 1193, 1928.

striction of the hepatic veins. In the earlier stages many lobules were flooded with blood, especially in their central portions. This stasis later disappeared almost completely and only an occasional lobule was found filled with blood. The intrasinusoidal cell masses so numerous in the first week also disappeared, for they were rare in the livers of those dogs which survived for periods of from fifty-two to sixty days. The average ratio of liver weight to body weight, although still above normal in group 2, was lower than that in Simonds and Callaway's series of animals. The hyaline thrombi in the central and sublobular veins also vanished with time. Swelling and granulation of the hepatic cells continued occasionally at least to the fifty-second postoperative day. But after the twenty-fifth day hydropic degeneration or edema of the hepatic cells was more common. We are unable to offer a satisfactory explanation for the continuance of this change.

In spite of these progressive or continued degenerative changes there was nothing resembling cirrhosis of the liver. The only increase in connective tissue occurred about the central veins where the mechanical effects of distention were most severe during constriction of the hepatic veins. This differs from the central fibrosis frequently observed in cases of chronic passive hyperemia of the liver. We have been unable to find this condition described in the literature. The process appears to begin between the fourteenth and twentieth days with an accumulation of large cells containing oval or indented nuclei immediately around the central veins. Later the central vein is found to be surrounded by a thick ring of connective tissue cells which are sometimes arranged radially as in figure 2 *A* and sometimes more or less concentrically. The radial arrangement appears to cause stenosis of the vein. When the connective tissue occurs in irregularly concentric layers, the vein is patent and spaces apparently for the passage of blood are present in the fibrous wall, as shown in figure 2 *B*.

SUMMARY

The livers of twelve dogs in which the hepatic veins had been mechanically constricted for periods of from ten to fifty minutes presented the following changes when examined from seven to sixty days after operation.

(a) Swelling and granulation of the hepatic cells as in parenchymatous degeneration were more frequently observed in the earlier stages but were still present in two dogs on the thirty-fifth and fifty-second postoperative days, respectively.

(b) Extreme swelling of the hepatic cells from either hydropic degeneration or intracellular edema, with apparently complete collapse of the sinusoids although occasionally present even before the seventh

day, was more characteristic of the later stages and was marked in two dogs on the sixtieth postoperative day

(c) The ratio of liver weight to body weight was greater in the second than in the first group

(d) The hyperemia, the dilatation of the lymphatics accompanying the sublobular veins and the intrasinusoidal cell masses, so prominent in the very early stages, gradually disappeared with lapse of time

(e) Fibrosis of the walls of the central veins was characteristic of the later stages. This change does not appear to have been previously recorded

In only one dog was there retention of bromsulphalein, and this was observed only once, twenty-four hours after the operation

The titer of the blood lipase was increased in five of eight dogs studied. The titer usually returned to normal by the fifth postoperative day, but in one animal it was still above normal on the fourteenth day

One dog was killed quickly with ether on the tenth postoperative day because of uncontrollable convulsions which could not be satisfactorily explained. Because of the presence of numerous "fatty infarcts" in the liver (also observed at the time of operation), it was thought that the convulsions were in some way related to hepatic insufficiency

Case Reports

ACUTE VALVULAR ENDOCARDITIS IN THE NEW-BORN

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The purpose of this paper is to record the microscopic picture of an active valvular endocarditis in the new-born. No attempt is made to explain its origin. After reviewing the literature on the subject, we have come to the conclusion that active fetal endocarditis must indeed be a rarity.

REVIEW OF THE LITERATURE

Ribbert¹ was unable to find that a fresh lesion of the ulcerative or verrucous valvular type had ever been seen in the fetus or in the new-born infant. He mentioned the cases of Fischer² and Kockel,³ and pointed out that the changes noted by them were old, consisting of thickening or shrinkage of the valves with no direct evidence of an inflammatory process. Ribbert's contention was that these changes might well be the result of an inflammatory lesion which had run its course, or that they might be congenital malformations of the valve, he believed that the latter was the more likely. Several further cases which are recorded in the literature fall into the same category, especially those reported by Loeser,⁴ Ludwig,⁵ Dissmann⁶ and Sawalischin⁷. In all these cases, no definite claim was made that fetal endocarditis was the cause of the changes observed. However, the possibility was suggested in each case.

Ayrolles,⁸ Boinet⁹ and Ganefff,¹⁰ in their case reports, gave fetal endocarditis as the etiologic factor in the changes seen. Ayrolles reported the case of a boy, born at term, weighing 2,790 Gm, who lived for four days, became suddenly extremely cyanotic, and died. The mother gave a history of chronic osteomyelitis of the left femur, which began to discharge actively again in the third month of pregnancy. Autopsy of the infant revealed a complete obliteration of the mitral

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1 Ribbert, H, in Henke, F, and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol 2, p 206

2 Fischer, B. Frankfurt Ztschr f Path **7** 83, 1911

3 Kockel, R. Verhandl d Gesellsch deutsch f Naturf u Aerzte **80** 39, 1908

4 Loeser, A. Virchows Arch f path Anat **219** 309, 1915

5 Ludwig, E. Cor-BI f schweiz Aerzte **42** 921, 1912

6 Dissmann, E. Frankfurt Ztschr f Path **43** 476, 1932

7 Sawalischin, K. Ueber angeborene Stenose des Aorten- und Mitralklappens infolge fetaler Endocarditis, Bonn, Emil Eisele, 1908

8 Ayrolles, P. Rev mens d mal de l'enf **3** 222, 1885

9 Boinet, M. Bull acad de med, Paris **53** 172, 1905

10 Ganefff, cited by Loeser⁴

ostium and a partly obliterated ductus Botalli. The mitral leaflets presented a light red discoloration, while fine adhesions, which were easily detached, were found between the chordae tendineae. No microscopic description accompanies the report.

The case of Boimet⁹ was as follows. A 23 year old woman, pregnant, was brought to the hospital because of cardiac disease, she was extremely dyspneic, had marked edema of the lower extremities and claimed to have had heart disease for the preceding three years following pneumonia. There were mitral insufficiency and pneumonia. The patient miscarried and was delivered of a dead fetus at approximately six months, the mother died, and autopsies were made on both. Pneumonia of the right lower lobe was found in the mother. The mitral valve of the mother showed marked fibrosis with stenosis of the ostium. There were several fibrotic nodules on the leaflets. The organs of the fetus were normal except for the heart, which showed a condition analogous to that found in the mother. The mitral valve showed a moderate degree of insufficiency, while several fibrous nodules, the size of millet seeds, were present on the leaflets. Again no microscopic description accompanies the report.

In the case reported by Ganef¹⁰ there were "thickening and shrinking of the aorta and a mitral insufficiency."¹¹ The endocardium was thought to have some localized areas of fresh inflammation. On microscopic examination, the endocardium consisted of dense fibers with few nuclei. The nuclei were seen to increase in number toward the musculature. There was a marked increase in connective tissue. This was considered true verrucous endocarditis and fibrous myocarditis although no distinct evidence of an acute inflammatory process was present.

Conclusive evidence of an existing or a former acute endocarditis seems lacking in each of the last three cases. We are inclined to place them in the same category as those already mentioned.

Capelli¹² more recently reported his observations in the study of sixty-two hearts of human fetuses. He did not encounter acute endocarditis in any case. The slight changes noted by him were either proliferative or degenerative. However, he found thrombi on some of the leaflets. These thrombi were extremely small, consisting of fibrin with a few nuclear elements. Capelli believed that the thrombotic deposits were caused by the changes already present in the leaflets of the valves, or by some toxemia rather than by bacteremia. Similar explanations have been given in the past by Vierordt,¹³ Rauchfuss,¹⁴ Dilg¹⁵ and others. They believed that valves with congenital malformations are points of predilection for thrombo-endocarditic lesions.

Abbott¹⁶ stated that "fetal endocarditis, which was believed by the earlier authors to play such an important part in the causation of cardiac anomalies, probably occupies a very minor role, being limited to those relatively few cases in which a rheumatic endocarditis is directly transmitted from mother to offspring." Abbott, however, does not mention any cases observed.

11 Shrinking of the aortic valve is probably meant.

12 Capelli, E. *Pathologica* **24** 103, 1932, *Sperimentale, Arch di biol* **87** 129, 1933.

13 Vierordt, H., in Nothnagel, H. *Spezielle Pathologie und Therapie*, Vienna, A. Holder, 1898, vol 15, p 225.

14 Rauchfuss, C., in Gerhardt, C. *Handbuch der Kinderkrankheiten*, Tubingen, H. Laupp, 1878, vol 4, p 132.

15 Dilg, J. *Virchows Arch f path Anat* **91** 193, 1883.

16 Abbott, Maud E., in Osler, W. *Modern Medicine Its Theory and Practice*, edited by Thomas McCrae, Philadelphia, Lea & Febiger, 1927, vol 4, p 627.

We were able to find record in the literature of just one such example which was proved by autopsy. The case was reported by Poynton¹⁷. His description reads: "The child of a mother, the victim of an attack of rheumatic fever in late pregnancy, died on the second day after birth of congenital heart disease. I found exuberant vegetations on the mitral valve forming a relative stenosis, and these vegetations contained numerous diplo-streptococci indistinguishable from the streptococcus rheumaticus, a condition of the mitral valve which pointed to an intra-uterine inflammation rather than a true arrest in development." No histologic description was given by Poynton.

REPORT OF A CASE

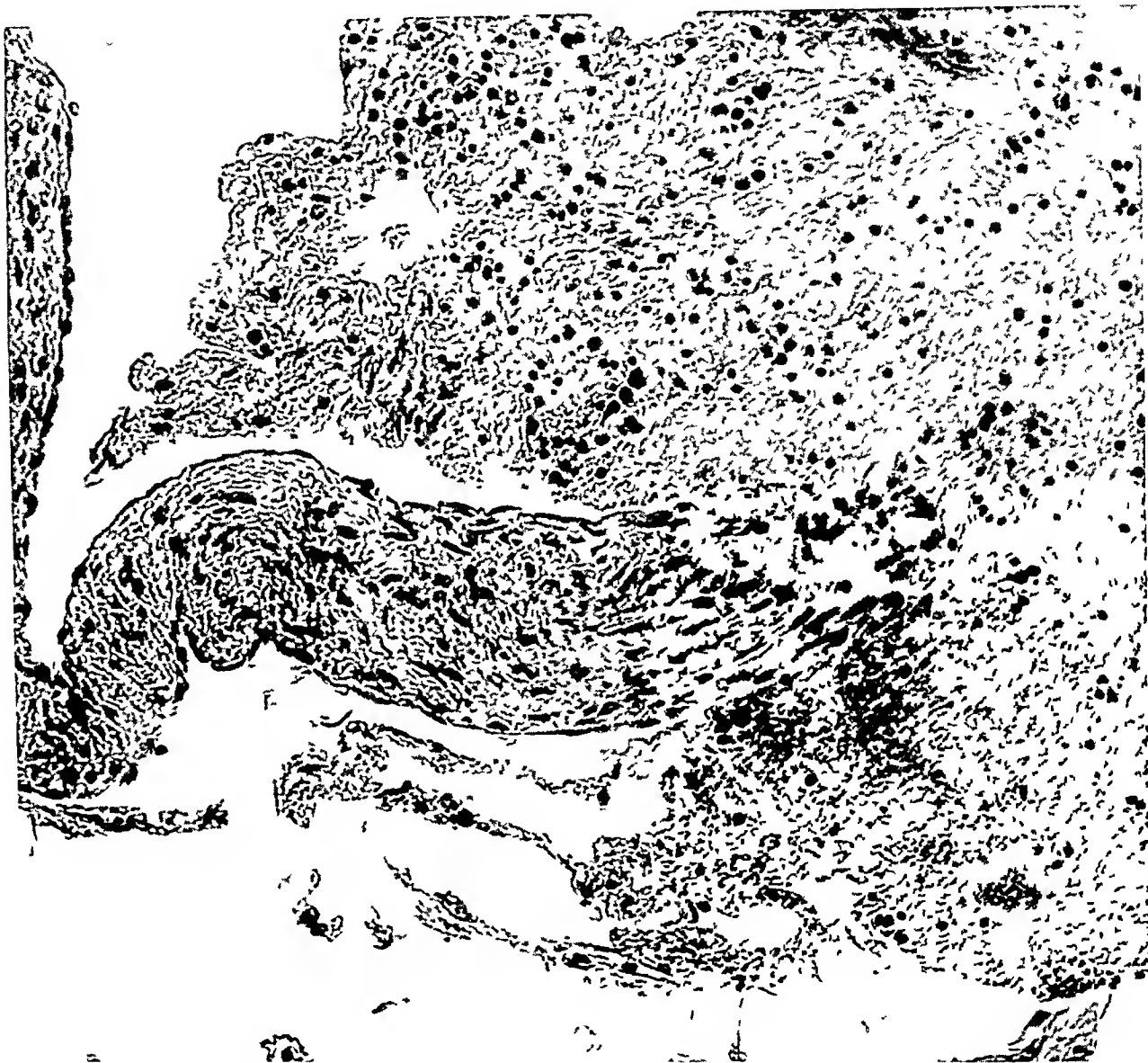
Our case is also one of a fresh verrucous endocarditis on an otherwise normal mitral valve. The lesion was found in a fetus from 6½ to 7 months old, that died one and one-half hours after birth. The mother gave a history of two previous pregnancies which ended in miscarriages, one of from five to six weeks, and the other of five and one-half months, both miscarriages were spontaneous. The medical history of the mother was essentially unimportant. The only mention of an illness elicited was that of a so-called mild influenza, which occurred from three to four weeks prior to delivery. A Wassermann test of the blood was negative. There were moderate tachycardia, tremor and enlargement of the thyroid gland. The delivery was normal. At birth the baby weighed 2 pounds and 9 ounces (1,162.3 Gm.), it was cyanotic and gasping, and died within one and one-half hours.

Autopsy revealed a normally developed female fetus, 38.5 cm. in length. The organs were all grossly normal. A small pinhead-sized, so-called valvular hematoma (blood nodule or sinus) was noted on the mitral valve. Because of a special interest in blood nodules, we sectioned the portion of the leaflet containing the nodule, in series. Approximately fifty sections were available for microscopic study, all stained with hematoxylin and eosin. The remainder of the heart was discarded. No special search was made for endocarditic vegetations on gross examination.

The microscopic study of these sections led to the accidental discovery of a fresh thrombus attached to the leaflet at a point definitely separated from the hematoma. The intervening space was approximately 0.2 mm. wide and showed no pathologic changes, the lining endothelium was intact and the connective tissue of the leaflet in this area showed no alterations. The vegetation could be studied only in the first few sections of the series. It measured 1 by 0.75 mm. on the slide, but we are unable to tell how large it may have been in its nonexamined portion. Unfortunately, no further material was available. The vegetation consisted of a fine fibrinous network which was diffusely permeated by polymorphonuclear leukocytes and different cells with medium-sized simple nuclei. Some of these nuclei stained very deeply. In addition to the diffusely distributed ones, there were a few densely packed groups of these cells, the densest group being situated near the point of attachment of the vegetation. The endothelial lining of the leaflet was missing at the point of attachment of the vegetation. The nuclei of the valvular tissue beneath the vegetation were swollen, the tissue was homogeneous and stained intensely with eosin. There was no evidence of cellular infiltration or of vascularization of the leaflet in this area. No bacteria could be found. (Only sections stained with hematoxylin and eosin were available.) The myocardium was normal.

¹⁷ Poynton F. J. Clin J. **34** 231, 1909

Microscopic examination of the suprarenal glands, lungs, liver and spleen showed nothing unusual. The hematopoiesis in the liver corresponded to the age of the fetus. Some deep red spherical areas were noted in the brain. They were formed by accumulations of undifferentiated germinal cells and did not represent an inflammatory process. The pancreas was histologically normally developed.



The vegetation consists of fibrin, leukocytes and mononuclear elements. The valvular tissue itself for the most part is normal. At the attachment of the vegetation, the connective tissue of the valve is homogeneous, and the nuclei are swollen and deep staining. At the lower edge of the picture, endothelial nuclei are seen protruding from the otherwise normal valve.

for the age of the fetus. However, it contained several large groups of round cells. The largest group of such cells included areas of pancreatic parenchyma and ducts. The other areas of lymphoid cells were well demarcated, lying in the interstitial

tissue, free from the pancreatic parenchyma, and seemed to be lymph follicles rather than an inflammatory tissue. They did not contain germinal centers.

COMMENT

In the presence of an acute inflammatory process on the heart valve of a new-born infant we must attempt to correlate these findings with other findings in the mother or the baby. The only illness of the mother during pregnancy, as mentioned, was a so-called mild influenza. There was no history of rheumatic fever. While, theoretically, one cannot exclude the possibility of a relation between the influenzal attack of the mother and the endocarditis of the fetus, there is no evidence to prove such a relation, or even to make it appear probable.

While we were searching for other inflammatory lesions in the organs of the new-born baby, our attention was caught by the bloody nodules in the brain and the groups of round cells in the pancreas. The cerebral lesion was definitely what older authors called Virchow's encephalitis. Pathologists are practically agreed today that these accumulations of cells are not inflammatory.

The accumulations of round cells in the pancreas might, at first, have been taken for an inflammatory process. However, Nakamura,¹⁸ who made a thorough study of the normal pancreas in ninety new-born and older infants, reported that the condition described occurred in 11 per cent of the cases. He maintained that it is not an inflammatory process but rather is linked to the condition known as status lymphaticus.

Any relation between the blood nodule (valvular hematoma) and the endocarditic lesion is highly improbable. First, the two lesions in our specimen were separated from each other by normal tissue. Furthermore, there have been numbers of careful investigations, with serial sectioning of valvular hematomas. None of these has been the seat of an inflammatory lesion (Wegelin¹⁹).

We are therefore unable to explain the genesis of this endocarditic lesion. We do not know how often such microscopic lesions may be present on the normal-appearing valves of the new-born. The study of Capelli seems to indicate that they are not frequent.

SUMMARY

A case of acute mitral endocarditis in a premature new-born infant is reported. Its origin cannot be explained. No other inflammatory process was found in the organs of the infant. Acute endocarditis in the new-born seems to be extremely rare.

18 Nakamura, N. *Virchows Arch f path Anat* **253** 286, 1924.

19 Wegelin, C. *Frankfurt Ztschr f Path* **9** 97, 1911.

CARCINOID OF MECKEL'S DIVERTICULUM

REPORT OF TWO CASES

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AND
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A carcinoid in Meckel's diverticulum is one of the rarest pathologic curiosities. In the literature we were able to find reports of only 2 cases. Hicks and Kadinsky¹ reported the first case in 1922, and Stewart and Taylor² reported the second case in 1926. After examination of the specimens, Stewart and Taylor expressed the opinion that there was a strong possibility that the lesion in the first case represented a heterotopia of gastric mucosa rather than a carcinoid. In the literature, besides the reports of the 2 carcinoids, we found records of only 8 sarcomas and 1 carcinoma of Meckel's diverticulum.

We wish to report 2 cases of carcinoid of Meckel's diverticulum which were found in a review of 6,138 cases that came to necropsy at the Mayo Clinic in the years 1923 and 1933 inclusive.

REPORT OF CASES

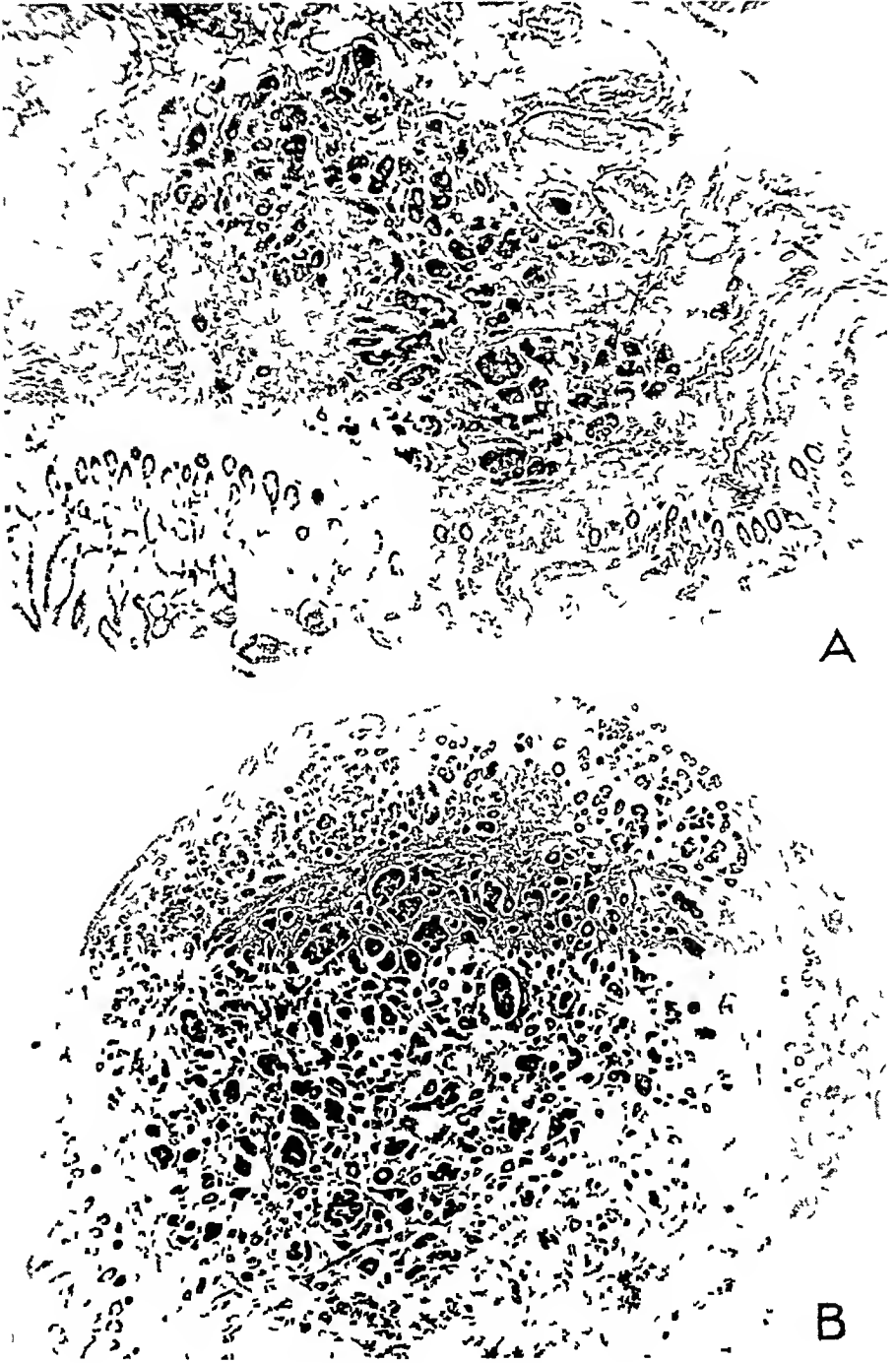
CASE 1—The patient was a man aged 54. A small irregularly shaped mass, 4 mm in diameter, was situated in the submucosa of Meckel's diverticulum (fig A). The overlying epithelium was similar to that of the ileum. The muscularis mucosae was fragmented. The tumor consisted of clusters of deeply staining spheroidal cells, separated by a dense connective tissue stroma. Higher magnification showed that the majority of nests consisted of densely packed hyperchromatic cells, each with a large rounded nucleus, which contained one or more nucleoli. The cellular outline was indistinct. The cytoplasm was granular and stained lightly with eosin. No mitotic figures were seen in any portion of the growth. Other nests of cells differed from the ones just described by the fact that occasional acinic formation was imitated.

CASE 2—The patient was a man aged 58. A small tumor, 3 mm in diameter, was situated at the tip of Meckel's diverticulum (fig B). The tumor was similar to that in case 1 in all respects, except that the cells had a tendency to assume more of a rough glandlike formation.

Work done in the Section on Pathologic Anatomy, the Mayo Clinic

1 Hicks, J A B, and Kadinsky, S. *Lancet* **2** 70, 1922

2 Stewart, M J, and Taylor, A L. *J Path & Bact* **29** 135, 1926



A, a carcinoid in the submucosa of Meckel's diverticulum, $\times 32$ *B*, a carcinoid in the tip of Meckel's diverticulum, $\times 25$

COMMENT

That these tumors were carcinoids is established by the typical histologic picture that has been described. The diagnosis was further confirmed by demonstrating the affinity of the cells of both tumors for silver salts.

The carcinoids were first described and separated from the carcinomas by Oberndorfer³ in 1907. Huebschmann,⁴ in 1910, suggested a relationship between the carcinoids and a type of granular epithelial cell of the intestine which had been described previously by Heidenhain,⁵ Nicolas,⁶ Kultschitsky⁷ and Schmidt.⁸ Oberndorfer,⁹ in 1909, and Gosset and Masson,¹⁰ in 1914, proved this relationship by demonstrating the similarity in staining with chrome and silver salts. Gosset and Masson gave the cell the name argentaffin cell because of this affinity for silver. Whether the argentaffin cell represents an entodermal cell which arises in the intestinal epithelium or an ectodermal cell which has migrated to the intestinal epithelium from the chromaffin tissues of the body is still an unsettled question. The argentaffin cells are found singly or in pairs among the columnar epithelial cells of the entire gastrointestinal tract. They are more numerous in the distal part of the ileum and in the appendix. Meckel's diverticulum is found in from 2 to 3 per cent of all cases which come to necropsy and is usually found about 90 cm proximal to the ileocecal sphincter. Although heterotopic tissue which simulates different portions of the gastro-intestinal tract occurs in Meckel's diverticulum, the diverticulum in the majority of instances is lined with epithelium which is characteristic of the ileum. Hence the occasional occurrence of a carcinoid is to be expected.

SUMMARY

The photomicrographs and histologic characteristics of 2 cases of carcinoid of Meckel's diverticulum are presented.

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- 3 Oberndorfer, Siegfried. *Frankfurt Ztschr f Path* **1** 426, 1907.
 - 4 Huebschmann, P. *Rev med de la Suisse Rom* **30** 317, 1910.
 - 5 Heidenhain, R. *Arch f mikr Anat* **6** 368, 1870.
 - 6 Nicolas, A. *Internat Monatschr f Anat u Physiol* **8** 1, 1891.
 - 7 Kultschitsky, N. *Arch f mikr Anat* **49** 7, 1897.
 - 8 Schmidt, J. E. *Arch f mikr Anat* **36** 12, 1905.
 - 9 Oberndorfer, Siegfried. *Ergebn d allg Path u path Anat* **13** 586, 1909.
 - 10 Gosset, A., and Masson, P. *Presse med* **22** 237, 1914.

PRIMARY GASTRIC LEIOMYOSARCOMA

REPORT OF TWO CASES

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Ewing¹ estimated that about 1 per cent of all gastric tumors are sarcoma. Edwards and Wright² expressed the belief that there are probably more cases of sarcoma of the stomach than the literature indicates, in an extensive review they were able to find reports of only thirty-eight cases of myosarcoma of the stomach. It is my purpose to put two cases on record and discuss them briefly.

REPORTS OF CASES

CASE 1—A white married man, aged 39, first admitted to the hospital on Dec 2, 1932, complained of pain in the epigastrium and weakness. Three years before he had diarrhea for about seven months, alternating with constipation and accompanied by gnawing epigastric pains from one to two hours after meals, which was relieved by food and alkalis. A week before admission the patient was seized with severe pain in the epigastrium which tended to radiate to the left hypochondrium and umbilical region. He felt marked weakness and dizziness but did not vomit. Examination disclosed only a marked epigastric tenderness where a hard mass was felt. There were marked secondary anemia, blood in the stool and normal gastric juice except for the presence of blood. Several transfusions were given, and the patient was discharged improved, with the diagnosis of gastric hemorrhage secondary to gastric ulcer or carcinoma. Two weeks later the patient was readmitted, complaining of dizzy spells, weakness and tarry stools. A smooth globular filling defect was found near the cardiac end of the stomach extending up from the greater curvature. The area of the defect did not show the normal rugae. There was one area about 1 cm in diameter which continually remained filled with barium as though it were an ulcer. After the loops of the small intestine became filled with barium, a large "vacuole" about 8 or 9 cm in diameter was found just below the greater curvature in close relationship to the gastric deformity. Exploratory laparotomy was made. Between the greater curvature of the stomach and the transverse colon and between the two layers of the omentum was a bluish mass, twice the size of the adult fist apparently a hemangioma. The tumor was adherent to the greater curvature by an isthmus continuous with an intragastric mass half the size of the extragastric tumor. No metastases were seen,

From the Department of Pathology, Isaac Kaufmann Foundation, Montefiore Hospital

1 Ewing, J. Neoplastic Diseases, ed 3, Philadelphia, W B Saunders Company, 1928

2 Edwards, C R, and Wright, R B. Am J Surg 19 442, 1933

and a partial gastrectomy was performed. The patient was discharged three weeks later after an uneventful recovery, with the hemoglobin 64 per cent and the red blood cell count 3,780,000.

The specimen consisted of a partially resected stomach with an attached tumor. The tumor was divided into intragastric and extragastric parts separated by a short pedicle. The intragastric mass was oval, measured 5 by 4 by 3 cm, was covered with normal gastric mucosa except one ulcerated area measuring 10 mm in diameter and 5 mm in depth, had a sharp margin, and was free from fibrosis or induration, the base was necrotic. The extragastric part, almost spherical, measured 12 cm in diameter, was bluish purple, soft and fluctuating, and was covered with peritoneum. When sectioned, the intragastric mass was well outlined and separated from the gastric mucosa, it was pinkish gray, soft and friable. The extragastric part collapsed after incision and consisted of multiple cystic cavities filled with hemorrhagic fluid and loose fragments of broken-down tissue.



Fig 1—The resected part of the stomach in case 1, showing the intragastric and extragastric masses and the ulcerated area in the mucous membrane.

Microscopically, the main bulk of the tumor was composed of interlacing bundles of smooth muscle cells, uniform in size and shape, with areas of whorl formation. Other parts revealed large granular cells, mostly undifferentiated short spindle-shaped, oval or round cells, the nuclei were massive and rich in chromatin. There were also single and multinucleated giant cells and a few mitotic figures. The stroma was poor in connective tissue, the blood vessels were ill-defined sinusoids, no definite layers of the vascular walls could be identified. The extragastric part showed cystic degeneration, the tumor cells were swollen and stained poorly. The gastric mucosa was normal, and there was no evidence of infiltration with tumor cells. The diagnosis was leiomyosarcoma of the stomach (intramural and subserous, undergoing degeneration).

CASE 2—An adult white woman, aged 32, was admitted to the hospital on Dec 4, 1934, complaining of fainting after having vomited a large amount of blood. On that day the patient awoke with a gnawing sensation in the epigastrium, which

persisted until the early evening and was accompanied by numerous diarrheic movements, with the feces abnormally dark in color. Shortly after dinner, she felt extremely dizzy and nauseated, began to vomit blood and fainted. The hematemesis was repeated twice before she could be brought to the hospital. For the past

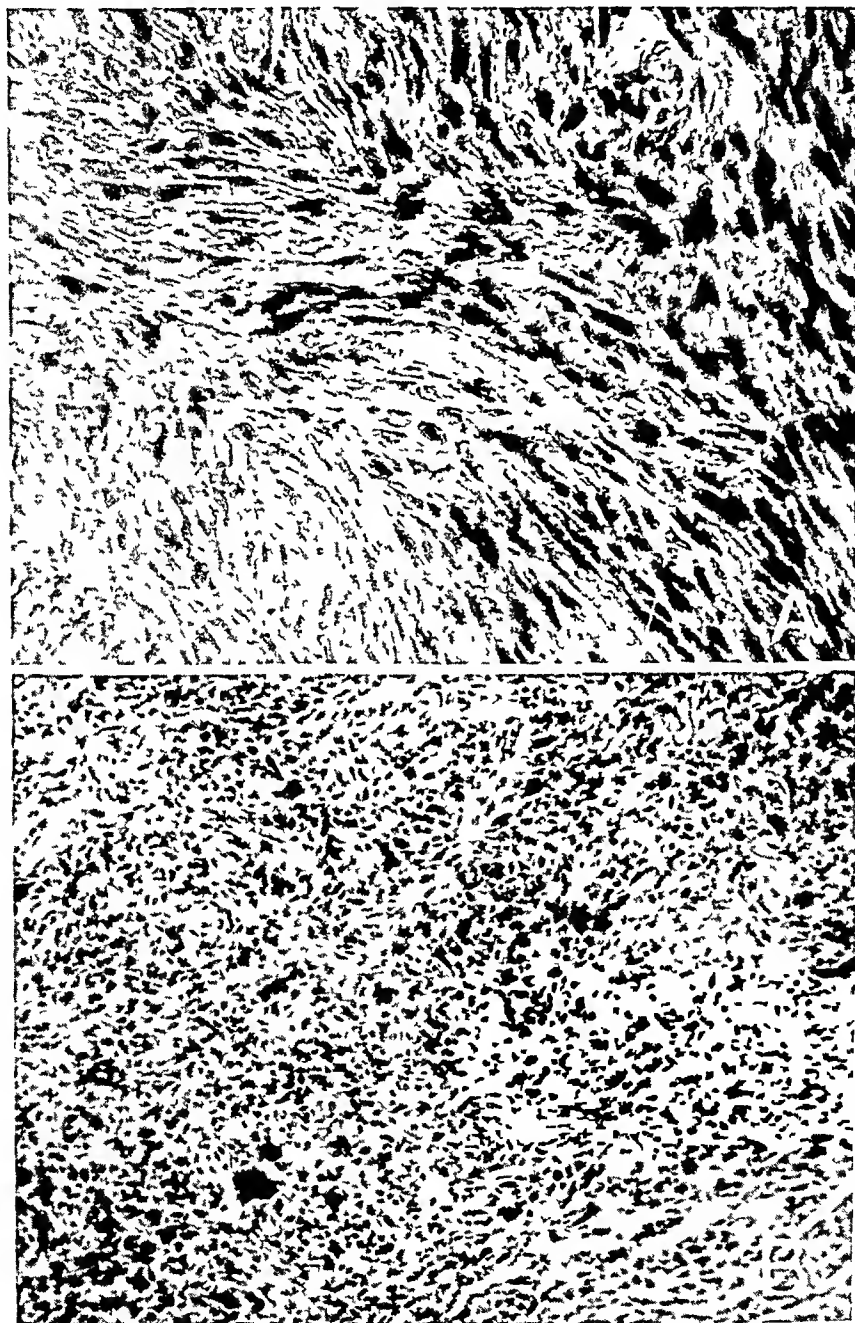


Fig 2 (case 1) —*A*, smooth muscle cells in whorl formation, $\times 450$ *B*, undifferentiated polymorphous cells, $\times 150$

nine years, the patient had had numerous gastro-intestinal complaints suggestive of disease of the gallbladder, a diagnosis which had been corroborated by a cholecystogram. Physical examination revealed only the appearance of shock, anemia

and obesity. No abdominal rigidity, tenderness or masses were apparent. The impression was that of gastric ulcer with hemorrhage and chronic cholecystitis and cholelithiasis.

The patient responded quickly to the usual treatment for shock. On the next day she received a transfusion of 500 cc of citrated blood, and during the next week there was steady improvement on a modified Sippy diet with appropriate alkalis. At this time a large filling defect on the lesser curvature of the stomach close to the junction of the pars media and the pars cardia was reported. This was

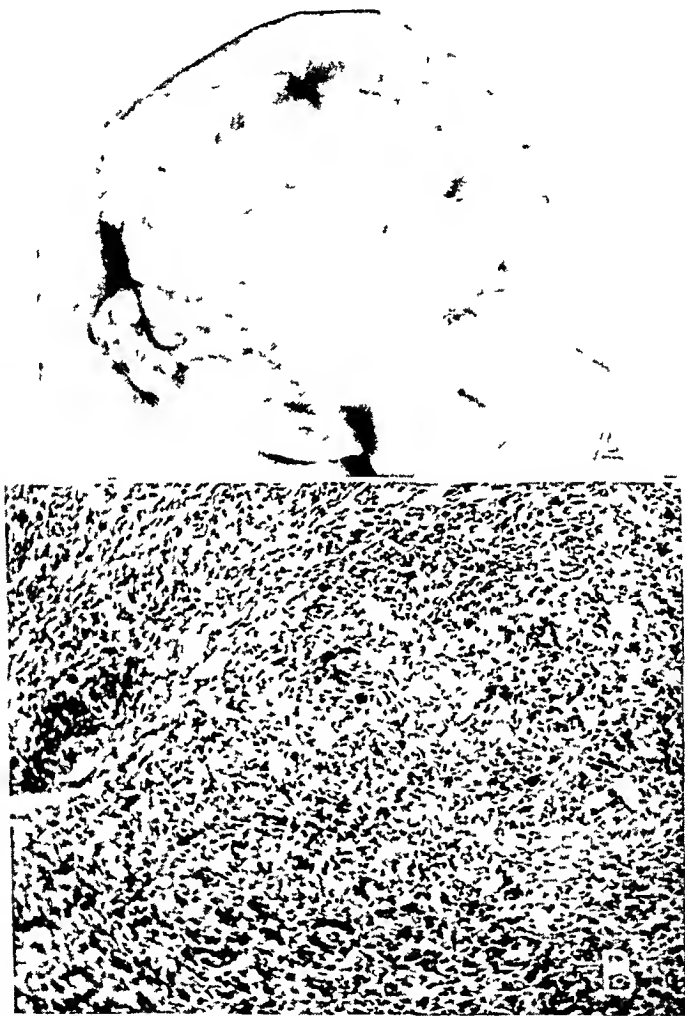


Fig 3 (case 2) —*A*, the tumor with ulcerations in the mucous membrane. *B*, atypical cells without definite arrangement, $\times 100$.

considered to be a large polypoid tumor of the stomach. A second transfusion was given, and an exploratory laparotomy was made. A tumor about the size of a golf ball situated on the anterior surface of the stomach midway between the greater and lesser curvatures was excised. The tumor did not appear to be malignant grossly. The patient was discharged two weeks later after an uneventful recovery.

The specimen was an oval tumor measuring 5 by 3 by 2 cm, most of the surface was covered with gastric mucosa, and a small area with peritoneum. There were

three ulcers through the mucosa, the largest being 5 mm in diameter and 4 mm in depth. At the base of one of the ulcers there was a vessel with an eroded wall. The rugae were well preserved and apparently free from infiltration. The tumor was encapsulated, the main part was intragastric, and the smaller part protruded under the peritoneal covering. The cross-section revealed a pinkish-gray, fleshy, homogeneous mass, soft and friable.

Microscopic sections revealed a cellular and vascular structure. Most of the cells were smooth muscle cells, long spindle-shaped, with oval nuclei. The cells ran in bundles in all directions. They were well differentiated and had the characteristic appearance of the cells of benign myoma. Other areas showed cells that were poorly differentiated and variable in size and shape. The cytoplasm was granular, the nuclei were large and hyperchromatic, with one or two large, deeply stained nucleoli. There were a few giant cells and occasionally mitosis. The stroma had little connective tissue, but the capillaries and blood spaces were abundant, dilated and filled with red cells. There was slight lymphocytic infiltration throughout the stroma. The gastric mucosa was normal and separated from the tumor by normal muscularis mucosae. The diagnosis was leiomyoma of the stomach (with sarcomatous changes).

COMMENT

The most common locations of gastric leiomyosarcoma are the curvatures of the stomach, it seldom involves the pyloric portion, and obstruction is therefore uncommon. The cardiac portion of the stomach was involved in none of the reported cases. The tumor varies in size from a minute growth a few millimeters in diameter to one so enormous that the entire abdominal cavity is filled. It is usually single but occasionally multiple.

Anschutz and Konjetzny³ divided sarcoma of the stomach into three classes from the gross point of view: intragastric, extragastric and infiltrating. It has been noted that leiomyosarcoma has a tendency to form intragastric or extragastric masses with pedicle formation, while lymphosarcoma generally infiltrates through the layers of the stomach. The cases now reported followed the general rule for the myosarcoma in their location. This type of tumor is well encapsulated and demarcated from the rest of the gastric layers, giving the appearance of an innocent tumor. Ulcerations through the mucosa are seen in many cases, as in these two cases, the first having a single ulcer and the second multiple ulcers eroding the big vessels of the submucosa with subsequent massive hemorrhage. Furthermore, the tumor has a tendency to softening with formation of cysts. Metastasis has been observed in a few cases. It is generally limited to the abdominal organs. The metastasizing cells may spread by invasion of contiguous tissues, by implantation on the omentum and various parts of the intestines and

3 Anschutz, W., and Konjetzny, G. E. *Die Geschwulste der Magens*, Stuttgart, Ferdinand Enke, 1921.

by carriage to the liver and distant organs. Kaufmann⁴ stated that the extragastric and submucosal varieties are of long duration and not of a high grade of malignancy. He believed that metastases occur in only one third of cases in which there is no operation, and that the most frequent site is the abdominal lymph nodes. The first patient, at the time of writing, two years and four months after operation, is free from symptoms of recurrence, and roentgen examination of the stomach and lungs shows no signs of it. In the second case only a few months have elapsed since the operation.

The determination of the origin of the tumor depends on the type of cells forming the main bulk. At times this is fairly simple and definite, while at others, because of marked anaplasia of the cells, it is doubtful. In both cases, as the photomicrographs show, there are areas that have the appearance of cellular myoma, while other parts show undifferentiated cells with hyperchromatic nuclei. This cellular myomatous appearance in many areas of the tumor has been the cause of controversy as to malignancy. Some authors hold that such tumors are benign and that no recurrence should be expected after extirpation, while others report cases of benign myomatous growths that have taken on malignant properties.

SUMMARY

Two cases of primary leiomyosarcoma of the stomach are reported. There were areas resembling benign myoma and also sarcomatous areas. Since primary leiomyosarcoma of the stomach still is a rarity in comparison with other neoplasms of the stomach, and as most of the cases on record are reports of observations at necropsy, cases diagnosed before death should be placed on record. The incidence of gastric sarcoma is in the ratio of from 1/100 to 1/300 of all gastric tumors. Myosarcoma tends to grow intragastrically and extragastrically and to undergo cystic degeneration and ulcerations, therefore hematemesis or melena is common. The consensus is that the type of tumor just reported has little tendency to metastasize and hence is the least malignant of the malignant neoplasms of the stomach.

⁴ Kaufmann, Edward. Pathology for Students and Practitioners. Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 692.

General Review

PATHOLOGY OF YAWS

ESPECIALLY THE RELATION OF YAWS TO SYPHILIS

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When one is endeavoring to trace the origin of syphilis, the relation of syphilis to yaws arises as a problem connected with, perhaps inseparable from, the original problem. In both infections there are a primary lesion and an ensuing state of generalized lesions, spirochetes of similar form occur in the early lesions of the two diseases, and in both the serum reactions (Wassermann, Kahn) become positive and the lesions usually clear up when arsphenamine and similar preparations are used. There are also differences between syphilis and yaws. My purpose in this paper is to discuss the resemblances and differences, particularly from the point of view of the structural changes. Comparisons of yaws with syphilis have been made many times but the changes in structure have usually been given less attention than the other aspects of the problem. The history of yaws will not be considered here, it may possibly be taken up in a later paper.

As yaws does not occur in the United States, many pathologists may not be as familiar with it as they are with syphilis. I shall therefore give certain facts concerning yaws as they appear in the literature, attempting at the same time to note points in which yaws and syphilis are alike or different. Attention will be called to references that give reviews of the extensive bibliography.

INCIDENCE, TRANSMISSION AND SYMPTOMS

Yaws is also known as fiambesia (on most of the continent of Europe), pian (in France and the West Indies) and bubas (in many Spanish American countries) and by other local names. It occurs almost exclusively in the tropics or in regions closely adjacent to the tropics. Prominent localities for it are Haiti and Jamaica and other islands of the West Indies, some parts of Central America and of tropical South America (chiefly Brazil and the north coast), various Polynesian and Melanesian islands, such as Fiji and Samoa and the Solomon Islands, Australia, New Guinea, Guam, the Philippine Islands, Netherlands Indies and notably Java and Sumatra, French Indo-China, the Federated Malay States, Ceylon, a few parts of British

India (chiefly Burma and Assam), Madagascar, and an immense territory in tropical Africa. Its absence among the Negroes of the southern parts of the United States is noteworthy (see extensive bibliography and history given by Wood).

Yaws is sometimes curiously uneven in its distribution, being present in a given locality and absent from neighboring localities for no apparent reason. The disease is occasionally imported into a country with a temperate climate through a seaport, such as New York, but the infection does not spread among the general population. Powell described graphically the dissemination of yaws through Assam from a single small focus of infection. Yaws is said to occur less frequently in towns than in rural districts, and that may be partly the result of treatment or of voluntary or enforced segregation of patients. At high altitudes in the tropics the lesions of yaws are likely to appear in warm, moist parts of the surface of the body (Lopez-Rizal and Sel-lards). It usually occurs in members of dark-skinned races. While cases in white persons have been described, they are rare. Yaws affects persons of the poorer classes more often than the well-to-do.

Children and young adults are most likely to show the early manifestations of yaws. Frequently several members of a family are affected at one time. By nursing at the breast an infant may be infected by the mother, or the nursing infant may infect the mother. Sexual transmission may occur but is rare. The ordinary mode of transmission is evidently contact with fresh lesions, often at points where there has been slight injury or a preexisting ulcer. The scanty clothing of the natives of tropical countries, the naked children, the bare feet and the little huts or cabins where many people are huddled together give plenty of chances for contact. It is common to see the sores of a native with yaws crawling with ants or flies, which the sufferer seems not to notice, and doubtless the infection is often spread in that way. In certain localities biting flies probably inoculate the victims. Spirochetes have been found on or in flies and mosquitoes that have been in contact with the ulcers of yaws (Baermann, Krum, Turner and Peat). Yaws has been known to follow vaccination with cowpox (Powell, Wilson). As with syphilis, the point of primary invasion may be overlooked or forgotten, or possibly there may not be any visible primary lesion (Sellards and his associates, 1926). If hereditary or congenital yaws occurs, it must be rare. Most observers have denied that it exists, though Butler (1930) expressed the belief that it does occur. Leon's careful researches seem to leave the question unsettled.

Yaws, like syphilis, may be divided for convenience into primary, secondary and tertiary stages, but the stages do not seem to be as well defined as those of syphilis. The incubation period is said to be about three weeks, but it may be shorter or considerably longer. In the early

stages of yaws there may be the general symptoms that are noted in most cases of acute infection of moderate degree, such as lassitude, pains in the bones and joints, sometimes fairly severe, and a slight rise of temperature. Often no constitutional disturbance is mentioned. Serum reactions, such as the Wassermann and Kahn reactions, become positive.

SPIROCHETES

Shortly after *Spirochaeta pallida* was discovered, Castellani observed a similar organism in the serum of the papillomas of patients with yaws. The nomenclature of the group of spirochetes is still in a state of confusion. The name *Treponema pertenue* is employed for the organism of yaws by most writers of recent date and seems to have been adopted by Castellani. As will be seen farther on, there are many cases in which it is difficult to determine whether one is dealing with yaws or with syphilis, some writers using the word *treponematosis* to designate those doubtful cases.

Spirochaeta pertenue, *Spirochaeta pallidula* or *T. pertenue* is similar to *S. pallida* morphologically, being well described and illustrated by Ashburn and Craig. A few observers have stated that differences in form or staining properties exist, but there now seems to be fairly general agreement that there are no constant differences. The spirochete may be found in papillomas of the first and second stages without difficulty in most instances. Dark-field illumination and various staining methods may be used as for *S. pallida*. Cultures have been secured in the same manner as for *S. pallida* but with great difficulty, not much seems to have been done in that direction. As it is doubtful that the spirochetes cultivated from patients with syphilis are the ones that produce that disease a similar doubt must prevail for the spirochetes cultivated from the patients with yaws. Hallenberger said that he secured cultures easily in horse serum and successfully inoculated two Negroes with the cultures.

PRIMARY STAGE

The initial lesion is known as the "mother yaw," *maman pian* and *madie buba* and by similar names in different countries. In Haiti it occurs on the foot or on the leg below the knee in a majority of cases (Wilson and Mathis), though it may arise on any exposed part, such as the lip, face, breast or hand. The lesion appears as a papule which enlarges and becomes moist, and the discharge and the cells may dry on the surface, forming a crust. The lesion may lose part of its epithelial covering and form an ulcer. It is said not to be indurated (Rat and many others). However Butler (1935) called attention to the fact that syphilitic chancres often lack induration or any distinctive gross characteristic. Extragenital chancres especially may lack characteristic

hardness (Hutchinson) The ulcer may be from 1 to 2 cm in diameter or larger It may heal promptly may persist into the secondary stage



Fig 1—Probable primary lesion of yaws on the right thumb of a Negro woman aged about 38, of Leogane, Haiti It was stated that "the lesion appeared one and one-half months before, after an eruption which occurred during washing There was no other eruption on the body" (Gatherings of women to wash clothes at a well or pond are important social functions in Haiti) The Haitian physicians made a diagnosis of yaws



Fig 2—Cross-section of tissue removed at biopsy from the lesion shown in figure 1 When stained by the Levaditi method it showed a small number of typical spirochetes among the disorganized epithelial cells, rather deep down Small numbers of gram-positive cocci were found among the deep epithelial cells The tissue was given to me by Dr Joseph Perrier, Port au Prince

or, according to some observers, unlike the chancre of syphilis may still be present in the form of a chronic ulcer in the tertiary stage (Wilson

and Mathis) The diagnosis of a primary lesion without other manifestations of yaws must be somewhat uncertain (figs 1 and 3)

Histologic Aspect—The histologic aspect seems not to have been studied in as many cases as that of the secondary papilloma of yaws



Fig 3—A woman aged about 20 who came to the Haitian General Hospital, Port au Prince, from a small village The *maman pian*, shown at the right, was on the inner side of the right leg, 7 cm above the malleolus and measured 4 by 3 cm It was said to have been present for three months It was slightly elevated and reddish, with some yellow crusts, and had the appearance of a chronic granulating ulcer Some pale, flat macules were present on the upper part of each arm and on the chest, said to be the remains of an earlier eruption that had disappeared spontaneously The main lesions had rounded borders, they were from 1 to 2 cm or more in diameter, elevated and covered with grayish-white crusts They occurred on the thighs and trunk and especially on the face The reticular appearance of the dried exudate on some of the papules on the face is unusual, I believe (possibly due to treatment) The skin close to the papules was usually a little more deeply pigmented than the general surface It was said that in films from the primary lesion, stained by Fontana's method, a few spirochetes were demonstrated with great difficulty, probably owing to the fact that the patient had an injection of arsphenamine five days before The woman complained of pain, limped and appeared languid There was a trifling rise of temperature Eighteen days later, the treatment having been continued, the eruption had entirely disappeared, leaving mostly pale areas, but sometimes an increase of pigment The patient appeared to be well The primary lesion was greatly reduced in size Like many of the illustrations shown in textbooks and articles, the picture represents an extreme and not an average case These photographs were taken by Mevs, Port au Prince, Haiti

A few observers (Hallenberger, Stitt, 1929) have stated that primary and secondary lesions have a similar structure. As the chance of syphilis has perhaps a more characteristic histologic picture than any other lesion of syphilis, it is unfortunate that there are so few studies on the primary lesions of yaws for comparison. To be convincing a primary lesion from a patient in whom typical secondary papules of yaws were already present or developed later would be desirable, also a comparison with extragenital syphilitic chancres would be important. I have studied sections from a probable primary lesion of yaws, but no secondary eruption was mentioned at the time the biopsy was made, and the patient was not seen again. The structure (fig 2) was the same as that of the secondary papules of yaws that I have seen: infiltration of the epithelium with polymorphonuclear leukocytes,¹ marked acanthosis, infiltration of the papillae and underlying connective tissue with lymphocytes, plasma cells and leukocytes, slight infiltration around the blood vessels, and doubtful slight multiplication of the endothelium of the small blood vessels. The changes in and around the blood vessels were distinctly less marked than those in sections of genital chancres available to me for study. Typical spirochetes were observed in sections stained by the Levaditi method, they were not numerous and were in regions where the epithelium was split up by the infiltrating cells.

SECONDARY STAGE

The secondary stage is usually regarded as presenting chiefly superficial lesions of the skin. As it is not characterized by any symptoms indicating specific involvement of the viscera, it is assumed that the viscera are not involved. I have not seen an account of an autopsy in a case of acute florid yaws. An opportunity for such an autopsy would be offered only in a case of most unusual severity (Baermann) or more probably in a case in which death occurred through violence or from some intercurrent disease. Reports on animals inoculated successfully with yaws on which autopsies have been made have not mentioned any important changes in the viscera.

The characteristic eruption of yaws is said to begin from two to eight weeks, or even considerably longer, after the appearance of the primary lesion. Allowing for odd and unusual cases, which will be referred to later, which occur in all diseases, and for those in which no secondary lesions are manifested, the eruption follows a pattern that is often described as "monotonous" in its regularity. Practically all observers agree that it is the one certain diagnostic feature of the disease. This monotonous eruption is different from syphilitic eruptions

¹ In the remainder of this article, for the sake of brevity, I shall call polymorphonuclear leukocytes simply leukocytes.

(Fox, 1929) except those syphilids that are called frambesiform, some instances of rupial syphilis and some condylomas, which are like the papules of yaws seen on moist parts of the skin

In his article on syphilis in the tropics Manteufel stated that secondary papular syphilids in the tropics may sometimes resemble the secondary eruption of yaws and that, on the other hand, the secondary



Fig 4—Lesions on the flexor aspect of the legs of a 13 year old girl with an extensive general eruption and a strongly positive Wassermann reaction Two younger brothers were similarly affected This photograph was given to me by Dr C M Hasselmann, Manila

eruption of yaws may at times resemble secondary syphilids (see also the article by Schuffner)

The eruption of yaws begins in the form of papules, which enlarge, often to the diameter of a centimeter or considerably more, and appear as moist papillomas Several papules may become confluent Exudate,

dead epithelium and leukocytes coat the surface with a yellowish or dirty white covering. When that layer is peeled off there remains a reddish granular mass, said to look like a raspberry (hence the name *frambesia* from the French word for raspberry). The name *poly-papillomas* is descriptive but is not often used (figs 3 to 5). These yaws are generally multiple, often widely distributed, rarely occurring on mucous membranes but frequent at mucocutaneous junctions.



Fig 5—A family of Madioen, Java, before and after treatment for yaws. The photographs were given to me by the Public Health Service of Netherlands Indies through Dr John L. Hydrick, of the Rockefeller Foundation.

Schobl (1928, p. 218) failed in his attempts to infect the nasal or vaginal mucous membrane of monkeys. The scalp is rarely involved. Paronychia occurs but is not common. The characteristic eruption may be preceded by a roseola (Schuffner) or by a fine desquamation at various points. Some observers have emphasized itching as a symptom (Rat, Manson-Bahr, Castellani and Chalmers, Jeanselme). Some have stated

that of a large number of papules produced metastatically, only a small part develop so as to become typical of secondary yaws (Rat, Sellards). Most writers seem to have held the opinion that the organisms causing the disease are distributed from the primary lesion by way of the blood stream. New points of infection apparently may arise also from the rubbing of an infected surface against an adjacent surface, as from one thigh to the other (Wilson). It is beyond the scope of this article to describe unusual departures from typical yaws, but annular or circinate lesions may be mentioned because of their comparative frequency (fig 6).

The papillomas of yaws may heal spontaneously, or they may persist for a long time (from two to six months or more, Schuffner). In healing they leave areas that are sometimes lighter and sometimes darker



Fig 6—A circinate form of yaws, so-called ringworm yaws, on a Negro girl aged about 6 years. The mother yaw appeared on the foot three months before, and the secondary yaws, a month later, according to the history. There were a few lesions about the vulva. No treatment had been given as yet. The clinical diagnosis of yaws was made by experienced physicians at Leogane, Haiti.

than the adjacent skin (Baermann). Under treatment with modern arsenical preparations (introduced for the treatment of yaws by Strong in 1910) the lesions disappear in a fashion that observers characterize as magical, dramatic or miraculous. Data are lacking that show whether or not these apparent cures are permanent, but the results may be called encouraging (Sellards, 1923, Lopez-Rizal and his associates, 1926, Moss, Baermann, Butler [1930], Lambert). In several localities a reduction in the prevalence of yaws following the treatment of large numbers of patients has been reported (Turner, Saunders and Johnston).

Lymphatic Glands—Most writers on yaws have given little space to the subject of the lymphatic glands. Rat referred to the enlarge-

ments that he saw as sympathetic and unimportant. Nevertheless enlargement of the regional glands has been mentioned by many as being common. The inguinal and epitrochlear glands have been referred to as being involved frequently, but other regions also may be affected.

Sellards, Lacy and Schobl observed that the regional lymphatic glands enlarged in from six to seven weeks after experimental inoculations in 6 human subjects. Harley found the epitrochlear glands palpable in 79.1 per cent of more than 5,000 cases of late yaws. Usually it has been stated that enlargement of the lymph nodes is slight, but in certain localities Lopez-Rizal and Sellards found enormous enlargement. It has been suggested that involvement of the lymphatic glands may be due in part to secondary infection (Moss and Bigelow, Wilson). In sections of an epitrochlear gland White and Tyzzer did not observe any marked change except hyperemia. The spirochetes have been demonstrated in the lymphatic glands in cases of active yaws (Ikegami, White and Tyzzer, Baermann, Schobl, in monkeys, 1928).

Lesions of Bones—Lesions of the bones have been described as occurring in the secondary stage. They will be discussed in connection with the tertiary stage.

Histologic Aspect of Secondary Yaws—All the descriptions that I have seen have apparently been founded on sections of lesions of the skin removed at biopsy (figs 7 to 9). The condition is usually characterized as a granuloma, with much proliferation of the epidermis and much emigration of leukocytes.

The outer layer of epidermis is thickened and of a hyaline appearance and is coated with dried serum and cells. The deeper, interpapillary part proliferates downward, the acanthosis is usually marked. The pigment may be diminished in amount or wholly lacking in the epithelium of the area affected (Kellermann Deibel and Elsbach, Hallenberger). Mitotic figures may be seen in the epithelial cells in material that has been well fixed and stained.

The corium is infiltrated with numerous cells—lymphocytes, plasma cells, spindle-shaped cells, large mononuclears and leukocytes. The descriptions do not lay much stress on the edema, the dilatation of blood vessels and the small hemorrhages that are sometimes seen, injury inflicted by removal of material for biopsy may be responsible for these results in part. Many observers have stated that the infiltration consists chiefly of plasma cells, especially in older lesions, and some even apply the name plasmoma to the condition. Others have not observed that plasma cells are especially conspicuous, and that has been my own experience. While there may be some perivascular arrangement of cells in the corium, the tendency of the cells to gather about the vessels is

not marked Endarteritis and periaarteritis are not more noticeable than in many inflammatory areas, in contrast to what is common in syphilis Giant cells are rarely seen In some cases eosinophils are numerous in



Fig 7—Cross-sections of one of the early papules on the back of a 10 year old Filipino girl before treatment was begun I saw this patient with Dr Chiuto at the San Lazaro Hospital, Manila She had had secondary lesions for about three weeks, a moderate number on the back and a few other scattered lesions The photographs were made from sections prepared by Dr Manalang, pathologist to the hospital Pigment was diminished in amount or was absent beyond the normal edge Leukocytes could be seen emigrating through the epidermis, though there were no actual abscesses The cells in the corium were lymphocytes and plasma cells, with some leukocytes next to the epithelium The blood vessels were not notably involved

the corium. As patients in the tropics are so often infested with intestinal and other worms, that fact might be noted in connection with the eosinophilia. Some form of allergic reaction might also be considered. Mast cells have been mentioned by some observers, but they do not seem to be important. New capillaries have rarely been mentioned.

Leukocytes are usually found invading the epidermis in large numbers, they may obscure the junction of the epidermis and corium, and they may be seen in all the layers of the epidermis in the process of migration. In some of my sections epithelial cells and exudate made a small homogeneous necrotic area, and when invaded by leukocytes this matrix looked curiously like cartilage or osteoid tissue (Gram-Weigert

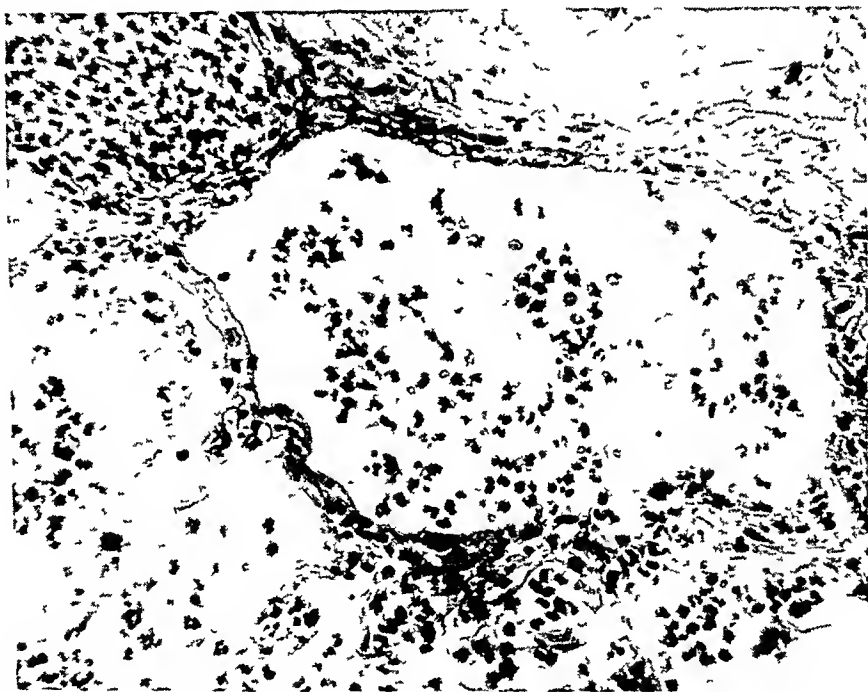


Fig 8—Section of a secondary lesion of yaws given me by Prof Gomez and Dr Santa Cruz, of the Philippine Bureau of Science, Manila, showing abscesses in the epidermis. In the deeper parts, not included in the figure, downward growths of epithelium, with considerable numbers of mitoses, were seen. The corium contained collections of lymphocytes and plasma cells, numerous eosinophils and many leukocytes next to the epithelium. The blood vessels were not notably involved.

stain), apparently new capillaries also invaded the mass. Small abscesses may form on the surfaces of the papillae, just below the epidermis, or abscesses may be observed in the layers of the epidermis when its cells become separated, forming cavities that are filled with leukocytes (fig 8). These abscesses may contain many spirochetes (fig 9). The spirochetes of yaws are said to be epidermotrophic, because most observers have found them chiefly in the epidermis

(Schuffner and many others) They are readily demonstrated by the Levaditi method in sections. In nine of the eleven secondary papillomas of yaws that I prepared by the Levaditi method, characteristic spirochetes were shown and occurred almost exclusively in the epidermis. Among collections of spirochetes one may see granules the appearance of which suggests an origin from disintegrated organisms. The neighboring cells may show evidences of degeneration or disintegration. It is difficult to say whether or not the organisms occur within cells.

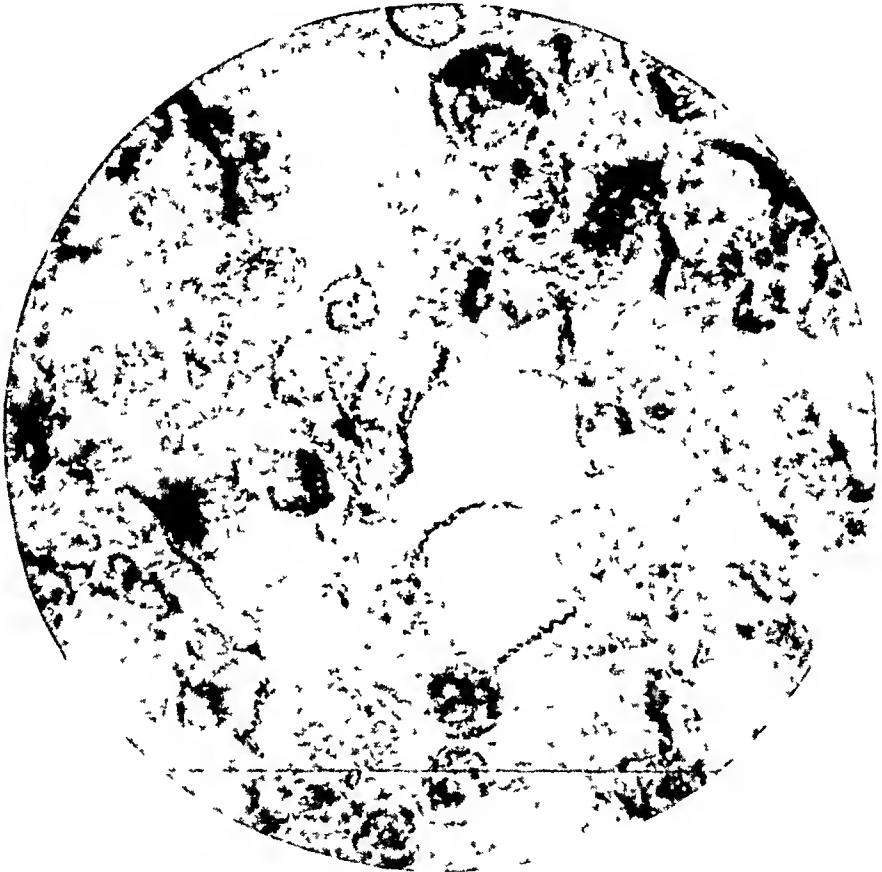


Fig. 9—Enlargement of a portion of a section from the lesion shown in figure 8 but stained by the Levaditi method. Part of one of the abscesses in the epidermis with numerous spirochetes is shown. It would be interesting to have similar abscesses examined also for pyogenic cocci by Gram's method.

In sections from a patient with an early stage of yaws, Goodpasture demonstrated spirochetes in large numbers in the perivascular tissue in the terminal portions of some papillae. He expressed the belief that the organisms were brought by the blood stream, and that the lesion began at that point and spread from there to the epidermis. Goodpasture also found that in sections of papillomas of yaws excised forty hours after the injection of neoarsphenamine and prepared by the Levaditi method no spirochetes could be demonstrated. He stated that

there was a great deal of destruction of leukocytes and much phagocytosis of leukocytes by large mononuclear cells in the same sections

In sections made in several recent cases and stained by the Gram-Weigert method I have seen good-sized colonies of gram-positive cocci and less often other organisms, usually just below the partly necrotic epidermis. It can hardly be doubted that secondary infection plays an important part in many cases of yaws, but evidence is lacking to show how much the microscopic picture is modified by secondary infection

Summary of Histologic Aspects of Secondary Yaws—The conception of the secondary papules of yaws that is derived from the work of various students is that the spirochetes usually reach the papillae of the skin by way of the blood stream from the primary focus or the regional lymphatic glands, probably in some cases direct inoculation from a neighboring part also takes place. By whatever route the organisms reach the starting point of the secondary growth, their multiplication proceeds chiefly in the epidermis. Downgrowth of the epidermis and migration of leukocytes into it are the outstanding features. At the same time new cells appear in the corium; practically all possible types of cells found in inflammatory areas are present except giant cells, which are rarely observed. The name plasmoma or even granuloma is somewhat misleading as it fails to suggest the significant involvement of the epidermis. The growth of the organism, chiefly in the epidermis, and the absence of marked involvement of the blood vessels are the most striking of the differences in the histologic picture of secondary lesions in yaws from those of syphilis. Syphilitic condyloma in particular, but also rupia and fiambesiform syphilids, may give pictures that closely resemble those seen in yaws. But, on the whole, investigators seem to have agreed that if a number of cases are compared, more involvement of blood vessels will be observed even in these forms of syphilis than in the secondary papillomas of yaws. This is an exceedingly conservative statement.

Histologic reports have been given by Fox, Hallenberger, Jeanselme, Kelleimann Deibel and Elsbach, MacLeod, Marshall, Schamberg and Klauder, Stitt (1929), Strong and Shattuck (1930) and White and Tyzzer. In their articles some additional references are given.

Crab Yaws and Clavus—Crab yaws and clavus, or clavos, are names given to certain lesions of the soles of the feet, crab yaws referring to the gait, which is thought to resemble the motion of a crab. Clavos is derived from the Spanish word for nails. There appear to be two conditions included under these names: (1) nodular lesions beneath the thick epidermis of the soles of the feet, which may break through to the surface, leaving ulcers or holes (Moss and Bigelow), and (2) hyperkeratosis of the epidermis, with cracks and fissures (fig. 10).

Many writers place these conditions, especially the nodular form, in the late secondary stage of yaws. To what extent the two conditions coincide is not clear from the literature (Schobl, 1928). The practice of going barefoot may have some influence on the hyperkeratosis. The palms of the hands may be involved in similar processes, but more rarely. If the lesions are of sufficiently early origin to contain living spirochetes, the serum exuding from them may offer one more means for spreading the infection. At rural dispensaries it is common to see several barefoot patients with lesions of the soles of the feet standing about the premises. At the station of the Rockefeller Foundation, Kingston, Jamaica, Dr. T. B. Turner told me that he has found



Fig. 10—Crab yaws on a patient at Kenscoff, Haiti (altitude of about 4,000 feet). Some of the white spots were pebbles lodged in the cracks in the epidermis. The clinical diagnosis was made by Haitian physicians.

motile spirochetes fairly regularly by dark-field illumination in the serum from the lesions of crab yaws. The histologic picture of the hyperkeratosis has been described by Gutierrez (1923). It has been said that inoculated monkeys sometimes show keratoderma closely resembling that seen in yaws in human beings (Schobl and Hasselmann).

TERTIARY STAGE

Disease of Bone in Yaws—For many years it has been alleged that the bones may be affected in late yaws, and a large number of observers in practically all parts of the tropics have alluded to this condition.

Some have denied that bone lesions are produced by yaws, and some still express doubt in this regard. Nodules resembling gummas, dactylitis, thickening of the bone proceeding from the periosteum and a sabie-shaped tibia are among the forms described. When thickening takes place beneath a chronic ulcer, it could be the result of a secondary periostitis and not directly due to yaws. The bone disease thus far described is not distinguishable grossly from that seen in cases of late syphilis. The argument advanced is that the disease of bone occurs in persons giving a history of having had yaws and showing the scars of previous yaws and that yaws is common in the district and syphilis rare or unknown.

Since the introduction of the roentgen rays more accurate studies are possible. It now appears that changes in the bones, which may be accompanied with considerable pain, may take place while papillomas of unmistakable active yaws are still present. Schuffner, in his report of cases in Netherlands Indies, was among the first to publish roentgenograms of the bones. The changes shown in his excellent roentgenograms could not be distinguished from syphilitic osteoperiostitis, while his diagnosis of yaws (in one instance of acute florid yaws) was of course conclusive. Soetomo and Eichorn, and Polak gave illustrations of other cases from Java. Maul described a series of patients seen in the Philippines and gave roentgenograms. In one case he published excellent photographs showing the primary lesion and unmistakable secondary papillomas and roentgenograms from the same subject. Maul's results differed from those of Schuffner in showing most commonly areas of rarefaction in the interior of bones, occasionally reaching the surface of the joint. In most cases there was one or a few of these areas, but one subject presented one hundred and thirteen. Several other processes, such as abscess, could produce similar pictures. Maul has found only a small percentage of cases of swelling of the surface or periosteal thickening.

At Kingston, Jamaica, in 1933 Drs. Turner and Saunders, of the Yaws Commission of the Rockefeller Foundation, showed me a large number of roentgenograms of children (fig. 11). Some of the children were said to have shown active lesions of the skin, in some, spirochetes were demonstrated in the lesions of the skin, others gave a history of having had yaws. According to my notes, the changes appeared in long bones and were of three types: areas of absorption, areas of absorption surrounded apparently by a region where there was new formation of bone and thickening of bones proceeding from the periosteum. The two latter types could not be distinguished from the changes produced by syphilis. The first type, simply showing areas of absorption, was the most frequent, the areas were from 0.5 to 2 cm. in diameter. Their

results will be published later, but I have been permitted to make this statement in advance

At Batavia, Java, Dr van der Plaats showed me roentgenograms of 7 patients for whom a clinical diagnosis of frambesia had already been made by other physicians. According to my notes, the films showed thickening of bone from osteoperiostitis like that of syphilis, areas like

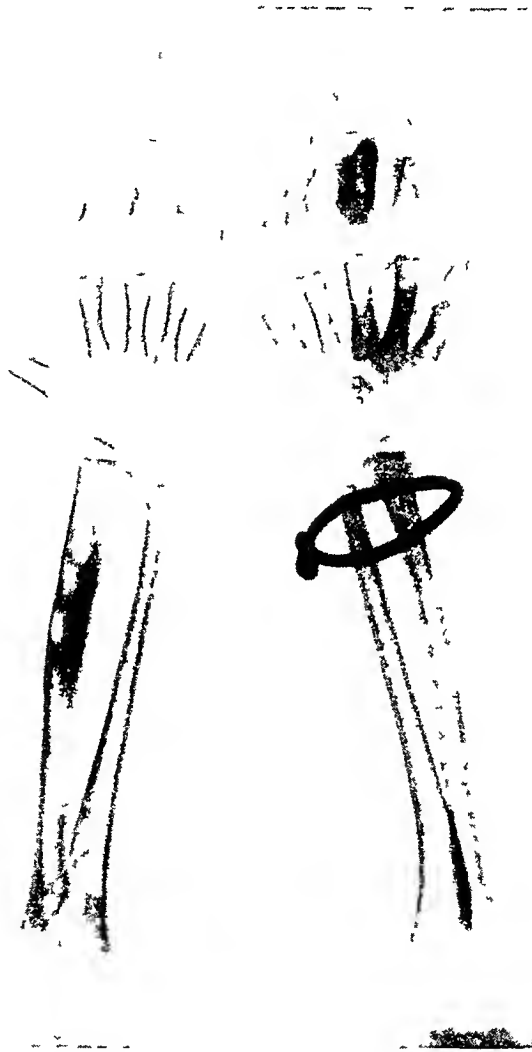


Fig 11—Roentgenogram of the hands and forearms of a 6 year old girl who had acquired yaws two years previously. She was given injections at that time, but osseous lesions and ulcerative cutaneous lesions developed a year later and persisted. This roentgenogram was made at the Kingston (Jamaica) Hospital and given to me by Dr T B Turner, of the Rockefeller Foundation. No secondary frambesiform lesions of the skin were then present, but Dr Turner had no doubt as to the diagnosis.

those produced by syphilitic gummas, and areas that might have been the result of osteomyelitis. Isek reported a case of early yaws in which frambesic papules and disease of the bones were present at the

same time Professor Ver Bunt, of Batavia, recently informed me that after reviewing the cases of patients with frambesia showing bone lesions who had come to his clinic during the preceding year, rather to his surprise it appeared that no patient showed osseous lesions simultaneously with the papulocrustate eruption, that is, the osseous lesions in this series were late lesions. Montel and Couput also described late lesions.

In general, observers seem to have agreed that the acute lesions of yaws in bones are commonest in children and that the bones of the hands, feet, legs and arms are most often involved. From what has been said it appears that some of the changes seen in roentgenograms are indistinguishable from those resulting from syphilis. However, the curious areas of absorption of bone, often multiple, are unlike the changes usually described for syphilis. They perhaps resemble some of the areas described by McLean in congenital syphilis of bone in young children. Direct action of the spirochetes, their toxins, allergy, secondary infection and mixed infection are among the possible explanations for the changes in the bones that suggest themselves. Histologic study of the bones in a series of these cases might furnish a solution.

Wolter gave a brief histologic description of a lesion in a case of dactylitis, apparently in a child (taken from Hashiguchi, whose work is not now accessible to me). There were thickening of the periosteum, with edema, slight infiltration of lymphocytes, plasma cells and fibroblasts, leukocytes were occasionally seen, especially in and about the walls of small veins with a more or less swollen endothelium. Proliferation of the capillaries, the smallest veins and the lymph clefts was observed. Necrosis and scarring were not demonstrated. Spirochetes were not seen. Wolter expressed the opinion that the dactylitis was similar to that of congenital syphilis but that it must be very rare in frambesia. He suggested allergy as an explanation. I do not know of any other histologic studies of the bones in yaws. Hermans described arthritis and involvement of tendons among the late results of yaws.

One point of difference from syphilis may be mentioned here. The involvement of the outer surface of the skull, especially the frontal and parietal bones, in a gummatous periostitis has long been regarded as frequent in severe cases of tertiary syphilis, but it is rarely seen today. The worm-eaten appearance of such a skull is well known. There is practically no evidence that yaws produces this type of skull. Sir Arthur Keith informed me of a skull of that type from the Society Islands, said to be that of a patient with yaws, but without other proof. Georg described a doubtful case from the Dominican Republic, which was probably a case of syphilis. In the literature I have not seen any report of the production of lesions of the bones in animals that had been inoculated successfully with the virus of yaws (with the excep-

tion of that of Hashiguchi quoted by Matsumoto) That includes the extensive series of monkeys infected by Schobl and the rabbits described in a recent report by Turner and Chesney Bone marrow from infected monkeys inoculated into other monkeys is said to have produced yaws (Neisser and his associates, quoted by Maul) In animals infected with syphilis, however, disease of the bones has been recorded many times

Late Ulcers—The difficulty or impossibility of distinguishing some of the late manifestations of yaws from the late lesions of syphilis has long been recognized In articles published in recent years there has been shown an increasing tendency to admit that certain lesions may be due to yaws that formerly would have been attributed to syphilis Some writers have considered that certain mutilating forms of syphilis that are alleged to occur in the tropics are really yaws The arguments for regarding these lesions as due to yaws are that in the communities in question primary venereal chancre and typical secondary cutaneous eruptions of syphilis are said to be rare, miscarriages among the women are rare and signs of congenital syphilis among the children are wanting, while yaws in the early stages is frequent

Chronic ulcers that cannot be distinguished from the late ulcers of syphilis seem to occur frequently in late stages of yaws Sometimes the ulcers arise from early lesions that have not healed, more often they appear first after an interval of months or years after the original attack They often occur on the leg and may originate in the breaking down of nodules in the skin that are like gummas The ulcers may heal after the destruction of a great deal of tissue and may lead to contractures and grave deformities I have not encountered any reference in the literature to the development of epithelioma on the late ulcers of yaws, and that must be an uncommon occurrence, I have learned of a few instances in personal communications

Dr H M Wade, of Cuhon, Philippine Islands, has sent me several photographs of patients with chronic ulcerative conditions, sometimes resulting in a great deal of scarring and deformity The lesions had been diagnosed incorrectly as leprosy and in Dr Wade's opinion probably represented late stages of yaws Specimens such as those illustrate forcibly the difficulties of diagnosis in many cases of chronic ulceration in tropical countries The same difficulties are well illustrated in the reports of Strong and others on work in Brazil and in Liberia (1925, 1930)

Gangosa—Gangosa and rhinopharyngitis mutilans are terms that are used more or less interchangeably The word gangosa, first employed in the island of Guam, is derived from the Spanish word meaning nasal voice In this disease there may be destruction of the soft parts of the nose and the adjacent parts of the face and of the palate There may

also be destruction of bone. Shocking mutilation may result, the process sometimes even extending to and destroying the eye. Gangosa commonly resembles types of late syphilis that formerly were seen in Europe and the United States but that have become rare at the present time. In localities where both syphilis and yaws are prevalent it may be impossible to make a positive diagnosis. In some instances gangosa is also said to resemble lupus, leprosy, blastomycosis and leishmaniasis. In some places where yaws is prevalent there may be many cases of gangosa, and in others, very few (Rat). Hunt and Johnson, who studied 2,000 cases of yaws in Samoa, stated that gangosa was excessively rare,



Fig 12—Gangosa (*rhinopharyngitis mutilans*) in a man aged 24. The soft palate but not the hard palate was perforated. I saw the patient several months after the photograph was taken, and there had been great improvement. The diagnosis was made by Prof Ver Bunt, Batavia, Java, who gave me this photograph.

if not unknown. That of course suggests that gangosa may be a manifestation of syphilis or perhaps of some separate infectious condition. Some writers have expressed the belief that the process originates in the mucous membrane, such as that of the soft palate, and that it extends from there outward (Stitt, 1929).

Schobl (1928) stated that in some of his monkeys inoculated with the virus of yaws he produced lesions of the nasal region closely resembling gangosa as seen in man. His explanation of the pathogenesis of gangosa is that in an infected animal an ulcer develops on the skin of or inside the nose, that the animal becomes allergic as a result of the infection and that finally the ulcer produces an intense

destruction of tissue, even including bone, extending from the skin to the mucous lining of the nasopharynx. Schobl expressed the belief that in some animals immunity develops, as will be shown later in this paper, and in such cases superinfection would fail.

Histologic Aspects of the Late Ulcers of Yaws and of Gangosa—Histologic study of the late lesions has received less attention than that of the papillomas of the secondary stage. Hallenberger and Strong and Shattuck (1930) each considered it at some length. What is found is practically the picture seen in any granulating ulcer, which may be more or less modified by the amount of secondary infection: necrosis and ulceration, infiltration of the underlying tissue with lymphocytes, plasma cells, leukocytes, fibroblasts and sometimes giant cells and new growth of capillaries. There may be a perivascular infiltration of "small cells." Spirochetes are said to have been demonstrated in several cases of gangosa by Baermann and others. Hallenberger stated that the histologic picture of the ulcers of late yaws is very much like that of comparable lesions in syphilis, the classic alteration of blood vessels in syphilis is the most important criterion for the differential diagnosis of its late lesions from those of frambesia. In a histologic study of 50 cases, he made a diagnosis of frambesia in 49 and of syphilis in only 1. But in old syphilitic conditions the characteristic periarteritis and endarteritis cannot always be demonstrated, as I have often seen myself. Also in simple chronic granulating ulcers considerable change in the vessels may occur. It appears, then, that in a series of ulcers due to yaws there would probably be less change in the blood vessels than in a similar series in cases of syphilis but that the diagnosis in a single case would be very uncertain.

Goundou—Goundou, or gundu, is the name given to a hyperostosis of the bones on the sides of the nose, sometimes likened to horns. Apparently it is seen most often in Negroes. Many observers have regarded it as a late manifestation of yaws, but others have not. Hallenberger stated that it is a peculiar thickening of the nasal process of the superior maxillary bone due to periostitis ossificans. He regarded yaws as its cause. In 1930 Strong and Shattuck published a report in which they discussed goundou and gave a review of the literature.

Juxta-Articular Nodules—Juxta-articular nodules owe their name to Jeanselme. They have been fully described by Strong and Shattuck (1930), who gave a complete bibliography on the subject. In the following account I have attempted to condense the facts, chiefly those given by Strong and Shattuck.

Juxta-articular nodules are firm subcutaneous nodular masses, usually situated near joints of the extremities. They may attain the size of an egg. Essentially they consist of layers of connective tissue

enclosing a central necrotic mass. The central part contains leukocytes and many large mononuclear foamlike phagocytic cells. In the connective tissue layers there may be some periarteritis and endarteritis. In the cases studied by Gutierrez (1925) the nodes consisted of cellular connective tissue. Most observers have not seen organisms in the nodes. Strong and Shattuck did not observe any organisms, though all kinds of technical methods were employed. A few observers claim to have demonstrated spirochetes (cited by Baermann). These nodules have been attributed to a fungus of the *Nocardia* group, to syphilis and to yaws. The filarial worm *Onchocerca* produces very similar tumors (Strong and Shattuck). Evidently, there are various causes for the production of nodes of this sort. Many observers have found them to be frequent in localities where yaws is common and have regarded them as a late manifestation of yaws.

Aneurysm—Many, if not most, writers on yaws have not mentioned aneurysm of the aorta. When it has been mentioned, the number of cases reported has usually been so small as to be without significance (Harley, Wilson and Mathis, Wilson, Hunt and Johnson, Macfie). However, Lambert stated that aneurysm and aortic disease are often seen in Fijians, among whom yaws is almost universal, while syphilis is almost unknown. The force of Lambert's statement seemed to me to be greatly lessened when he said that syphilis is frequent in East Indians and other Asiatics who have come to the same islands. A discussion of conditions in Fiji with regard to the occurrence of yaws and syphilis is found in connection with the papers of Manson-Bahr, Stannus and Powell.

The most extensive work on aneurysm and disease of the aorta in possible cases of yaws was that of Choisser in his report on 700 autopsies made in Haiti, which I shall summarize briefly. Most characteristic of yaws, Choisser stated, are patches of fatty degeneration of the aorta, beginning in the intima about 5 mm above the valves, and in linear scars, and sometimes extending so as to involve the entire aorta in extensive atheroma. The valves of the heart are not often involved. He noted 8 cases of aneurysm of the aorta, 1 case of gumma of the brain and 1 of cerebral hemorrhage. Opalescent patches in the pericardium and endocardium, a flabby myocardium and small scars in the liver were noted. Choisser also stated that spontaneous cerebral hemorrhage (4 cases) is not infrequent in young adults with clinical histories and symptoms of yaws, but he did not describe the symptoms.

In estimating the significance of Choisser's studies, one should inquire whether or not he could possibly have been certain that he was dealing with yaws. He said that his patients had "a negative history of syphilitic infection and no evidence of scars on the genitalia." Referring to the changes of the aorta he said that "the only other clue to the infection is the scar of the mother jaw." Concerning some of his

patients with aneurysms he said that they gave histories of yaws in childhood or years previously. The evidence consisted, therefore, of statements found in the clinical histories, in the absence of genital scars and in the presence of scars attributed to a previous attack of yaws. If any of the subjects showed the characteristic papillomas of yaws while under observation, Choisser did not mention it. His autopsies were made at the Haitian General Hospital, Port au Prince, which is a seaport where syphilis is conceded to prevail. The evidence that the aneurysms and other lesions described by Choisser were due to yaws does not seem to me to be convincing (see the article by Koltes and Albrecht on the prevalence of syphilis in Haiti).

Weller has recently studied the aorta in 169 cases at the Haitian General Hospital which Chambers selected from what seemed to be probable cases of "treponematosiis". One hundred and eleven specimens (66.8 per cent) showed histologic lesions similar to those of syphilis. Ninety-six of these were stained for spirochetes, and positive results were obtained in 28 cases.

The significance of these results depends entirely on the reliability of the clinical histories and on the importance of old scars of the genitalia in the diagnosis of syphilis and of old scars on the surface of the body in general in the diagnosis of yaws.

In a small number of these cases (11) there was a genital scar with a history of syphilis but not of yaws. In another small group (14) there were scars due to yaws with a history of yaws but not of syphilis, and no genital scars were noted. Nine of the syphilitic patients and 11 of those with yaws showed the microscopic changes in the aorta that are considered to be characteristic of syphilis. The unusual frequency of aortic aneurysm was noted. Weller did not consider that his study offered proof of either the unity or the duality of yaws and syphilis. An account of this work will appear in the *Transactions of the Association of American Physicians* for 1935. Dr. Weller has kindly allowed me to read an abstract of the paper and to publish this note in advance.

Other Conditions—*Tabes dorsalis* and *dementia paralytica* are not mentioned in many reports on yaws, in many other cases the rarity of these diseases in regions where yaws is prevalent is noted. As far as I can learn, nowhere but in Fiji has yaws been assigned as a frequent cause of these conditions (Lambert). The remarks on conditions in Fiji made in connection with aneurysm of the aorta apply also in this paragraph. However, these forms of neurosyphilis are said by some to be decidedly rarer in colored races than in white races. The possibility that the frequent malaria of the tropics exercises a restraining influence on their development and the possibility that neurosyphilis may be due to a strain of spirochete with special affinities for the nervous

system suggest fields for study of uncommon interest, but not included within the scope of this paper. Diseases of the eye and alopecia are not usually mentioned as being frequent in cases of yaws.

INOCULATION

Human Beings—Infection of human beings with yaws has been done with success by numerous experimenters by inoculation with material derived from patients with yaws. The results are cited by Ashburn and Craig and by many other writers.

Monkeys—In monkeys inoculated with material from papules of yaws of human beings which contained the spirochetes, local lesions usually developed that were somewhat like those seen in human subjects and that also contained spirochetes. Inoculations were made subcutaneously or by applying serum to an abrasion. Secondary lesions seemed to occur rarely (see Ashburn and Craig, who also cited the previous work of Castellani and of Neisser and his colleagues), but Baermann saw secondary lesions repeatedly. The monkeys used were animals of the genera *Semnopithecus*, *Macacus* and *Cynomolgus* and a few Gibbons and orang-utans. The incubation period varied from about fourteen days to more than ninety days. The spirochetes might be present in the spleen and lymphatic glands (Castellani) and in the bone marrow (Neisser and others). Numerous other investigators have made successful inoculations in monkeys (Nichols, White and Tyzzer, Schamberg and Klauder, Schobl and his colleagues, Hoffmann). The reports of autopsies as recorded for infected monkeys have not included mention of lesions of the internal viscera (Ashburn and Craig, Schobl). In the course of a large number of inoculations of monkeys (*Cynomolgus*) in Manila, Schobl (1928) rarely saw spontaneous metastatic lesions other than those occurring through the local lymphatic vessels. However, he was able to produce distant metastases by superinfection. The metastatic lesions that he produced were not as numerous or as extensive as those that are often seen in human beings. In general Schobl found these monkeys more resistant to yaws than man, and the lesions healed more promptly. Spirochetes were detected in the regional lymphatic glands only in cases of active yaws and not always in them. He saw no evidence that a latent infection existed in the lymph glands, but the spirochetes survived dormant in the skin. He stated that several types of lesions seen in man were reproduced in his monkeys, as has been mentioned with regard to hyperkeratosis and gangosa.

Rabbits—Rabbits were used successfully for inoculation with the virus of yaws first by Nichols and subsequently by Reasoner, Brown and Pearce, Jahnel and Lange and Matsumoto and his colleagues. Numerous experiments were reported recently by Turner, Chambers

and Chesney, who gave references to the work done previously. They obtained successful inoculations with material from 8 different patients with undoubted clinical yaws in Haiti. At the same time rabbits were successfully inoculated with material from a Haitian Negro with a typical hunterian penile chancre, this and six other strains of syphilis from sources in the United States and Europe were used for comparison with those obtained from the rabbits infected with yaws. It was found, as others had previously claimed, that yaws was more difficult to propagate than syphilis. In rabbits the average incubation period was longer in yaws (thirty-eight and six-tenth days). As Brown and Pearce had already noted, and as was corroborated by most though not all observers, the inoculated testicle showed scattered miliary granules in its body, in the tunic or the epididymis, the miliary lesions consisted of large and small lymphocytes and many eosinophils. Spirochetes were demonstrated in the active lesions, which lasted several weeks and slowly retrogressed. The character of the results remained the same with the different strains and with the lapse of considerable time, indicating that there was no modification of the virus by repeated passage through rabbits (in one strain through fifteen generations of rabbits). The strain of syphilis recovered in Haiti gave results in the testicles of rabbits like those seen with other strains of syphilis, with great enlargement and induration, and the number of positive results from inoculation was much larger than with yaws. A considerable portion of the rabbits inoculated with yaws gave negative or slight reactions. Occasionally a rabbit gave a reaction as severe as that seen in the rabbits inoculated with syphilitic material. In rabbits intracutaneous inoculation with the virus of yaws produced erythema, desquamation or enlargement of the papillae, owing to collections of lymphocytes, not like the button-like lesion produced in syphilis of the rabbit's skin. Inoculations in the testis produced metastasis to the uninoculated testis less often than was the case with syphilis. None of 87 animals inoculated with the virus of yaws and carefully observed showed lesions indicating generalization of the infection in the bones, periosteum, skin and eyes, whereas lesions of that type were seen in rabbits inoculated with the Haitian strain of spirochetes of syphilis. Hashiguchi, however, according to Matsumoto observed lesions of the skin, bone and eyes in a fair proportion of rabbits inoculated with the virus of yaws.

Manteufel and his colleagues stated that in the beginning the strain of organisms of yaws with which they worked produced changes in the testicles of rabbits that were notably milder than those produced by inoculation with the organisms of syphilis. But gradually their strain of organisms of yaws came to produce results like those seen in rabbits with syphilis (large masses in the testicle, deep, hard chancres).

except that the rabbits with yaws after healing of the manifest lesions only exceptionally showed inoculable virus in the glands, while it was almost always shown by rabbits with syphilis

Using a strain of organisms of yaws obtained in Sumatra, Hoffmann produced in rabbits a granular periorchitis like that described by Brown and Pearce and nodules containing numerous spirochetes. He observed unilateral nodular infiltrations consisting of lymphocytes and plasma cells involving the adventitia and media and even the intima of the small veins, he considered that this condition differed from the phlebitis of syphilis

Some Japanese investigators have stated that yaws can be transmitted to guinea-pigs by inoculation, the prepuce being the most favorable location. It is said that the local lesion is different from that produced by inoculations with the virus of syphilis (Tan and Ogiuti) and also that mice and rats may be inoculated with the spirochetes of yaws, and that the organisms may be demonstrated in some of the viscera for a long time thereafter (Misaizu)

IMMUNITY TO YAWS

Rat has said that second attacks of yaws occur but are rare. He also stated that in places where yaws is endemic "it generally attacks children, and experience shows that the disease runs a milder course than in the later years of life. So convinced of this are parents in certain parts of Africa that every facility is given to contract the disease in infancy, and even inoculation is resorted to in order to ensure this." Sellards said that "at Yamasa in the Dominican Republic the parents make a practice of freely exposing children to the disease because they feel that the sequellae, especially clavos, are less likely to be severe when the disease develops during infancy." Mattlet observed that near Lake Tanganyika in Africa mothers inoculate infants with material from persons with pian to ward off tertiary complications, in infants the secondary eruption is excessively florid. According to Stitt (1928), inoculation to procure immunity is practiced by natives of Guinea, and Manson-Bahr said that this has long been done in Fiji. In Java I met physicians who informed me that the natives believe that an attack of yaws confers immunity and other physicians who had not heard of any such belief. Among those who have had large experience with yaws the belief seemed fairly general that immunity is developed. Hermans cited numerous examples of this belief.

There are also reports of cases in which reinfection or superinfection has occurred, though it happens rarely (Baermann)

Sellards, Lacy and Schobl showed that volunteers could be infected with yaws. From four to six weeks later and while secondary lesions were present, they were again inoculated, and granulomas resulted

Sellards and Goodpasture (1923) and Lacy and Sellards (1926) observed that in subjects who had had yaws some months or years previously (some of them having had treatment) reinoculation sometimes gave positive results, more often abortive lesions or no lesions at all. These experiments indicated that the development of immunity requires a long time. Baermann cited experiments by Castellani, Neisser and others that seemed to show that monkeys that are successfully inoculated acquire immunity against yaws, he also considered and discussed the possibility that an apparent immunity may be due to latent infection.

Continuing the studies for which their inoculation of monkeys with the virus of yaws laid the foundation, Schobl and his colleagues performed an enormous amount of experimental work on immunity against yaws and syphilis, using several hundred monkeys. Their work had the merit of having been done in the tropics as well as having been conducted on an animal as nearly related to man as possible. The results were published in various numbers of the *Philippine Journal of Science* from 1928 to 1931. They were summarized in the paper of Schobl and Hasselmann.

Schobl stated that in monkeys inoculated with the organisms of yaws a positive (Wassermann, Kahn) reaction of the blood serum might develop (Garcia). A strongly positive reaction developed when the local lesion was marked and after repeated outbreaks in the animal, when there were slight local lesions or late lesions, the reaction was likely to be weak. The serum reaction was manifested in an early positive phase after the first inoculation, then a negative phase and then a second positive phase, whether more lesions appeared or not.

A condition resembling immunity might ensue, as was indicated when a fresh inoculation with infectious material was followed by a negative result. Immunity in inoculated monkeys usually appeared about six or seven months after the original inoculation. (The slow development of immunity may explain the conflicting results of other observers.) Schobl observed that the condition of resistance to superinfection was effective in proportion to the intensity of the early lesions in yaws and the number of invading organisms. He expressed the belief that his results were in harmony with the principles established by Brown and Pearce for experimental syphilis, which he stated as follows: "The number of the invading treponemas during the early stage stands in direct proportion to the degree of immunity that subsequently develops. It stands in inverse proportion to the time necessary for the development of immunity." The immunity endured for a long time, probably throughout the life of the monkey, it might persist after the serum reactions became negative. Schobl prepared a vaccine consisting of killed parasites from experimental lesions of yaws.

in monkeys. Injections of that vaccine produced in monkeys positive reactions of the serum the strength of which was in proportion to the number of organisms introduced. Schobl and his colleagues stated further that monkeys given injections of the vaccine were made immune so that they resisted inoculations with the living organisms of yaws.

CROSS-IMMUNITY BETWEEN YAWS AND SYPHILIS

Various observers from widely separated points have expressed the opinion, based on clinical studies, that infection with one of these diseases confers a certain amount of immunity against the other (Wilson and Mathis in Haiti, Manson-Bahr and Lambert in Fiji, Parham in Samoa and Connolly in East Africa), the more common opinion is that an attack of yaws sometimes gives immunity against syphilis. However, the evidence is somewhat conflicting (Mattlet, van den Branden and Dubois, Powell). MacCallum related a curious incident: "St. Johnstone examined carefully a labor battalion of Fijians which was sent to France during the war. They all had yaws but no syphilis. On their return there were many cases of gonorrhea, but none of syphilis." Some critics have regarded the reports from these islands as unconvincing and have questioned that the native population is as nearly free from syphilis as is alleged. It is known that the East Indians and Chinese in Fiji have syphilis (Powell, Stannus). Strong and Shattuck, and Baermann have given good summaries of what was known of the immunologic relations of yaws and syphilis up to 1930. In the present review I shall refer only to part of the work published. Several observers have reported the occurrence of one of these diseases in persons who had already had the other, for instance, Goodman saw yaws in a child who also had hereditary syphilis. Castellani, and Neisser and his colleagues found that monkeys that had been infected with yaws were refractory to yaws but susceptible to syphilis. Levaditi and Nattan-Larrier were successful in infecting monkeys with yaws but were unable to transmit yaws to monkeys which had previously been infected with syphilis. Nichols inoculated a small series of rabbits with yaws, and part of them were treated. After intervals of from ninety days to more than a year they were inoculated with an active strain of syphilis. More than half of the animals proved refractory to the second inoculation. About 25 persons with dementia paralytica were inoculated by Jahnel and Lange (1928) with a virus of yaws from infected rabbits using strains from America and from Sumatra. The results were negative in all but 1 case (Sumatra strain), in which an abortive lesion resulted. Jahnel and Lange have given a good summary of the characters of yaws and its immunology as reported up to 1928. They discussed the properties of different strains of the virus

of yaws and the time factor in the production of immunity. They suggested that yaws and syphilis express the extremes of a group of closely related viruses in which endemic syphilis, which is usually of extragenital origin, may be an intermediate process.

Manteufel considered some of these experiments and others and reviewed statements to the effect that a strain of syphilis has been said to protect animals from inoculation only with the same strain (Kolle and Schlossberger), that strains of organisms of yaws and of syphilis that did not have comparable degrees of virulence have been used in cross-immunity experiments and also that strains of the virus of yaws seem to acquire greater pathogenicity with repeated passages (see the discussion under inoculation of rabbits). Manteufel then expressed the opinion that experiments in cross-immunity do not give convincing evidence for or against the nonidentity of yaws and syphilis.

Schobl expressed the belief that fundamental differences exist between yaws and syphilis and that the two diseases may coexist in one animal. Nevertheless he noted a certain amount of both serologic and immunologic reciprocity between them, and he agreed that biologically the organisms belong to the same group. He and his colleagues experimented with monkeys, testing them in various ways with the Nichols strain of syphilis obtained from infected rabbits. This strain inoculated into the skin of the eyebrow or scrotum of the monkey gave a slight local reaction, soon healing, while the regional lymphatic glands continued to harbor viable organisms (tested by inoculation of rabbits in the testis). It is impossible to give here more than an indication of the kind of experiments made, for details the original articles must be consulted (Schobl, Schobl and Hasselmann). Schobl stated that monkeys that had been rendered immune against yaws by inoculation with living organisms of yaws "or by the injection of the lifeless vaccine [referred to previously] proved refractory to inoculation with syphilis a year later, and some gave earlier, slight or no local reaction and showed in the lymphatic glands no viable organisms, provided that the immunity from yaws infection had been given sufficient time to fully develop." From other experiments carried out on similar lines it was concluded that monkeys that were first inoculated with syphilitic material became resistant to subsequent inoculation with the spirochetes of yaws. In these tests the time factor and the amount and kind of material injected were of fundamental importance. Schobl concluded that a reciprocal immunity existed that was a group immunity against yaws and syphilis and that a homologous immunity also existed that developed earlier than the heterologous group immunity. One strain of yaws that was employed continuously for six years and repeatedly passed through monkeys kept its characters unaltered.

Schobl also noted that the strain of syphilis (Nichols) continued to produce a local sclerosis in monkeys as long as it was used

Dr Schobl has kindly examined and corrected the foregoing account of the work of himself and his colleagues Obviously his statements regarding his later experiments suggest possibilities of immense importance It is to be hoped that these experiments will be repeated and amplified, strains of yaws and syphilis from new sources being employed

Certain Japanese investigators reported that they failed to find evidence of cross-immunity between yaws and syphilis Matsumoto and his colleagues stated that rabbits might be superinfected with yaws within seventy-six days of the first inoculation, while superinfection usually failed eight months after the first infection But in animals infected with yaws that received thorough treatment with arsphenamine, reinoculation gave positive results twenty months after infection They observed that syphilitic rabbits were usually susceptible to inoculation with yaws Rabbits that had been cured of syphilis with arsphenamine were usually susceptible to yaws Six of 10 rabbits that were not susceptible to superinfection with syphilis (even with a heterologous strain) were still susceptible to inoculation with the organisms of yaws Kato infected rabbits with yaws, they were then superinfected three times and were found to be immune against yaws Thirty-one days after the third superinfection, spirochetes of syphilis, in part highly diluted, were introduced into the back The inoculations were said to have given uniformly positive results, with more or less prolongation of incubation Rabbits were infected with yaws and after from one hundred and two to three hundred and eight-nine days were cured with arsphenamine Twenty days later these rabbits were inoculated intravenously with an emulsion of syphilitic virus, and later all of them presented generalized lesions due to syphilis More recently Misaizu, using rats and mice, first infected the animals with the spirochetes of yaws, then treated them with arsphenamine and later reinjected them with the spirochetes of yaws He observed no evidence that specific immunity was produced by the first inoculation Such experiments as were performed to test the development of cross-immunity between yaws and syphilis gave negative results

SUMMARY AND DISCUSSION

A few years ago I began examining the evidence bearing on the relations of yaws and syphilis and had the advantage of being without any preconceived opinion It soon became clear that some acquaintance with the appearance of yaws was necessary, and I visited the tropical islands of Haiti and Java and the Philippines (Manila) I saw a

moderate number of patients with yaws (from 300 to 400), mostly on a single occasion only. Practically every type of lesion that I have seen described was observed, but there were only a small number of instances of obvious bone disease. I was deeply impressed with the fact that yaws is only one of many problems confronting physicians in the tropics, along with dysentery, beriberi, tuberculosis, smallpox, leprosy and sometimes bubonic plague and with malaria and worms always to the front.

Acute yaws is comparatively easy to manage by treatment, though what the final results of the treatment will be cannot yet be determined. The natives, having observed the results of treatment, appear at the clinics voluntarily in large numbers and bring their children. Patients with acute yaws seem not to come to autopsy. Autopsies in the tropics can be made with modern technical methods only at hospitals in large cities, where it is difficult to exclude the possibility that syphilis may be present.

Beginning with Rat's monograph and even in earlier treatises, yaws and syphilis have been compared many times, the points of resemblance or difference often being arranged in parallel columns. Most of these points have been mentioned in the preceding paragraphs. Because of a lack of large experience with yaws and with dermatologic diseases in general, I have probably made some mistakes. In this following summary, I shall confine myself chiefly to a discussion of the pathologic anatomy and the histologic picture.

The primary lesions of yaws and of syphilis differ decidedly in their histologic features, according to the small amount of information available. Final conclusions cannot be made until more material is at hand.

The secondary lesions of acute florid yaws differ decidedly in their gross appearance from most secondary eruptions of syphilis, though syphilis rarely causes lesions like those of yaws, and yaws is said sometimes to cause eruptions like those of syphilis. Histologically, typical lesions of yaws are characterized by the presence of spirochetes, chiefly among the epithelial cells, by marked proliferation of the epithelium downward, by the large number of leukocytes that penetrate the epidermis and by the slight amount of involvement of blood vessels. Thus, the papules of yaws differ from most syphilitic lesions, but occasionally condyloma and some of the cutaneous lesions of syphilis may give fairly similar pictures.

Late ulcers of yaws and those of syphilis are so much alike that a diagnosis between them is frequently impossible. It has sometimes seemed to me that in certain tropical regions similar conditions were likely to be called syphilis in the city and yaws in the country. Such information as is available indicates that the differences in the histologic

picture are not marked enough to be decisive in many if not most cases. However, further study must be made before a definite conclusion can be reached.

As to involvement of bone, it seems to me that it must be admitted that roentgenograms show that the bones are often affected in yaws and that the pictures are often like those seen in cases of syphilis. As far as I can learn there has been little histologic study in these cases.

The evidence that has been presented to show that aneurysms of the aorta are caused by yaws appears to me to be unconvincing. It is probable that the internal viscera in general are not involved in yaws, but the autopsy evidence is insufficient.

Both clinical and experimental work indicate that an attack of yaws confers considerable immunity against a second infection.

Also, clinical observations and laboratory experiments suggest that a certain amount of cross-immunity between yaws and syphilis may be produced, but the statements are somewhat conflicting. Possibly some of the discrepancies seen in experiments on animals may be due to the use of infectious agents having different degrees of virulence or to the fact that the immunity tests were made later in some experiments than in others. More work is urgently needed, preferably on monkeys, in my opinion, and in a tropical climate.

Opinions on the relationship of yaws to syphilis differ widely. Yaws has been called syphilis of the tropics and stone-age syphilis, syphilis and yaws have been called brother and sister as well as twins. Castellani regarded them as wholly distinct infections, like tuberculosis and leprosy. Butler expressed the belief that yaws is syphilis. An ingenious theory was suggested recently in a monograph by Essed. Syphilis was an old disease in Europe, yaws was imported into Europe from America by the sailors accompanying Columbus, it apparently has disappeared from Europe and has reappeared from time to time under various names, some endemic syphilis is really yaws.

More than forty years ago Hutchinson remarked that physicians practicing in the tropics almost always consider yaws and syphilis to be different diseases. I have discussed the matter with nearly fifty physicians in the islands, previously named, and my recollection is that almost without exception they recognized differences between yaws and syphilis, only a small number were noncommittal.

Apparently no one has recorded having actually witnessed the transformation of yaws to syphilis or of syphilis to yaws in man. Convincing evidence could hardly be secured except under the conditions of a laboratory experiment. Endemic syphilis, as observed by von During Pasha in Asia Minor, in an unusually degraded population resembles yaws in some particulars, but resemblance does not constitute identity. There is abundant evidence to show that Negroes in the

tropics may have syphilis of the ordinary form (van den Branden and Dubois), except that tabes and dementia paralytica are usually said to be rare. Probably all will agree that the spirochetes of yaws and syphilis originally had the same spirochete for an ancestor. The evidence that is available gives me the impression that the spirochete of either yaws or syphilis has undergone a functional but not a morphologic mutation in some human host, giving rise to the other infection, and that the resemblances between the two infections indicate that the new infection has evolved from the older one in comparatively recent times.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc—William H. Park, director of the bureau of laboratories of the health department of New York City since the establishment of the bureau in 1894, has been awarded the Roosevelt Medal for 1935 for "distinguished service in the administration of public office"

In Georgetown University, G. J. Brimlyer has been appointed professor, and I. A. Simpson and H. V. Connerty assistant professors, of pathology and parasitology

The Trudeau Medal of the National Tuberculosis Association has been awarded to L. U. Gardner in recognition of his work on tuberculosis, particularly the relation between tuberculosis and silicosis

Esmond R. Long, director of the laboratory of the Henry Phipps Institute of the University of Pennsylvania, has been made director of the institute. Charles J. Hatfield, formerly director, will be associate director and chairman of the board of directors. Henry R. M. Landis will have charge of the clinical and sociologic departments

Marion Dorset, biochemist in the United States Department of Agriculture, well known for his work on serum against hog cholera and author of many contributions of value to the livestock, meat and dairy industries and to public health, has died at the age of 63

An oil portrait of Milton C. Winternitz, who has retired from the deanship of the medical school of Yale University after fifteen years' service, has been presented to the school by the student body

Oswald T. Avery, of the hospital of the Rockefeller Institute for Medical Research, has received the honorary degree of LL.D. from McGill University

Morris Rakieten has been appointed assistant professor of bacteriology in Long Island College of Medicine, Brooklyn

Harry Goldblatt has been promoted to professor of experimental pathology in Western Reserve University, Cleveland

Gustav Ruediger, formerly professor of pathology in the University of North Dakota, director of the Hygienic Institute at LaSalle, Ill., and director of the hygienic laboratory of the University of Nevada, has died in Pasadena, Calif., of tuberculosis at the age of 59

Sanford V. Larkey, librarian and assistant professor of medical history and bibliography in the medical school of the University of California, has been appointed librarian of the Welch Medical Library of Johns Hopkins University, succeeding the late Fielding H. Garrison

Herbert S. Gasser, professor of physiology in Cornell University Medical College, has been appointed director of the Rockefeller Institute for Medical Research to succeed Simon Flexner, who will retire

Frederick F. Russell has resigned as director of the International Health Division of the Rockefeller Foundation and will be succeeded by Wilbur A. Sawyer, associate director. Dr. Russell will be lecturer on preventive medicine and hygiene at the Harvard University Medical School

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

PAROSMIA IN TUMOROUS INVOLVEMENT OF THE OLFACTORY BULBS AND NERVES
H A PASKIND, Arch Neurol & Psychiat **33** 835, 1935

A woman aged 45, three years after an operation for carcinoma of the breast, complained of headache, dizziness and reeling gait with a tendency to fall to the left. In addition, she continuously smelled an unpleasant odor for two weeks and had left homonymous hemianopia for three days. A carcinoma of the left cerebellar lobe was diagnosed and removed. Necropsy revealed carcinomatous masses throughout the central nervous system and viscera and, in addition, a carcinomatous nodule in each olfactory bulb. It also invaded the olfactory nerves, some of which were swollen or degenerated. Paskind emphasizes the extreme rarity of continuous parosmia caused by a tumor of the olfactory tract. He cites only three other instances, in which the disturbances of the sense of smell were due respectively to carcinoma, meningioma and glioma involving the olfactory tract bulb.

GEORGE B HASSIN

THE METABOLISM OF ISOLATED SURVIVING TISSUES FROM ANIMALS RENDERED
HYPERTHYROID WITH THYROXINE D McEACHERN, Bull Johns Hopkins
Hosp **56** 145, 1935

Representative tissues isolated from hyperthyroid animals continue to show increased consumption of oxygen. The increase in tissue respiration is only roughly proportional to the increase in total consumption of oxygen by the intact hyperthyroid animal. The increase of tissue respiration is not so great as would be expected from the increase of metabolism in the intact animal. The increase in the respiration of hyperthyroid tissue is not carried on by any new or abnormal respiratory mechanism in the cell. An increase of tissue glycolysis is not the fundamental cause of the increased consumption of oxygen. Cyanide, fluoride and mono-iodo-acetic acid reduce the respiration of hyperthyroid tissues but do not affect the fundamental mechanism which makes an increased supply of oxygen necessary to the organism. Iodine, when added directly to the tissue, has no specific effect on the increased respiration due to hyperthyroidism. Hepatic and renal tissues from hyperthyroid animals have no lessened capacity to oxidize various substrates (lactate, pyruvate, succinate). Hyperthyroid muscle has a greater capacity to oxidize these substrates than has normal muscle.

FROM THE AUTHOR'S CONCLUSIONS

THE TESTIS HORMONE C R MOORE, J A M A **104** 1405, 1935

The principal sources of testicular hormone at present are the testicles of large mammals and human urine. The hormone is obtained from the lipid fraction and has been sufficiently purified to yield crystals of high potency. It appears chemically to be a ketone-alcohol, the only known method of detecting its presence is by the reaction produced in suitable animals. It is secreted continuously (or periodically) in different animals, and the secretion is largely under the control of the pituitary gland. It is uncertain whether more than one hormone is secreted by the testis. Its clinical use is questionable, its primary function is the control of the accessory reproductive organs, it is not a testicular stimulant.

FROM THE AUTHOR'S SUMMARY

ACUTE EXPERIMENTAL STOMATITIS IN DOGS WITH LEUCOPENIA D K MILLER
and C P RHODES, J Exper Med **61** 173, 1935

An ulcerative stomatitis associated with leukopenia and granulopenia can be induced in dogs by means of a diet causing black tongue. The decrease in the number of circulating leukocytes is due to a suppression of the maturation of the erythropoietic elements of the bone marrow. The changes as a whole have a resemblance to those occurring in human beings with acute agranulocytosis.

FROM THE AUTHORS' SUMMARY

Pathologic Anatomy

REACTION OF PULMONARY TISSUE TO LIPIODOL R D WRIGHT, Am J Path
11 497, 1935

The reaction to iodized poppy-seed oil 40 per cent retained for long periods in the bronchi is the development of lipophages from the supporting connective tissues. No epithelial reaction occurs.

FROM THE AUTHOR'S SUMMARY

INFARCTION OF THE LIVER I J PASS, Am J Path **11** 503, 1935

A review of the literature on infarction of the liver with a report of two additional cases is given.

FROM THE AUTHOR'S SUMMARY

BASOPHILIC DEGENERATION OF THE HEART MUSCLE M E HAUMEDER, Am J
Path **11** 535, 1935

A peculiar lesion of the heart muscle is described under the term "basophilic degeneration." The most frequent sites of the lesion were the septum, the left ventricle and a combination of the septum and the left ventricle. Staining reactions showed that the areas contained mucin as well as a component related to glycogen. Hematoxylin and eosin was the most valuable stain because it clearly differentiated the areas of basophilic degeneration.

FROM THE AUTHOR'S SUMMARY

HEART BLOCK DUE TO CALCAREOUS LESIONS OF THE BUNDLES OF HIS W M
YATER and V H CORNELL, Ann Int Med **8** 777, 1935

Of the forty-seven cases of complete heart block authentically reported in the literature nine were due to fibrocalcareous or calcareous lesions of the bundles of His. Such calcium depositions were commonly seen at the base of the aortic (anterior) leaflet of the mitral valve, extending out into the membranous portion of the interventricular septum. They occurred late in life—no cases in persons below the age of 50, five instances in those in the eighth decade of life. All the patients had Adams-Stokes attacks. Sclerosis of the coronary arteries did not parallel the local lesion nor was there evidence of fibrosis of the myocardium except in two instances. By serial sections the authors demonstrated that a bar of calcium deposit in the usual location in the heart of an army officer who also had a syphilitic aneurysm of the aorta actually replaced or invaded all of the bundle of His. It was concluded that such deposition of calcium is due to stress and strain, since the main mass of the heart hangs from the area of the membranous septum. The bundle of His above and below the point of injury was found to be normal, demonstrating the non-nervous structure of this system.

FRANK R MENNE

THE CELLULAR REACTIONS TO ACETONE-SOLUBLE FAT FROM MYCOBACTERIA AND STREPTOCOCCI K C SMITHBURN and F R SABIN, *J Exper Med* **61** 771, 1935

The acetone-soluble fat of tubercle bacilli, when injected into normal animals, produces a profound cellular reaction. The reaction involves every type of connective tissue cell. Hemorrhage and formation of adhesions and of tuberculous tissue occur. The extent of the reaction is roughly proportional to the amount of material injected. The reaction induced by the lipid is much less extensive and much simpler when the material is neutralized with alkali. Neutralization of the acetone-soluble fat, or of phthioic acid, does not diminish the tuberculogenic property. Acetone-soluble fat from streptococci is likewise extremely irritating but does not produce tuberculous tissue.

FROM THE AUTHORS' SUMMARY

RUPTURE OF THE RENAL PELVIS BENJAMIN S ABESHOUSE, *Surg, Gynec & Obst* **60** 710, 1935

From a review of 64 cases from the literature and three from his own records Abeshouse concludes that rupture of the renal pelvis proper, traumatic or spontaneous, is relatively rare. Traumatic rupture is usually linear and radial. The external trauma may vary from a crushing injury to an indirect blow. Spontaneous rupture nearly always occurs in a kidney already the seat of chronic pyelonephritis or in one the pelvis of which is dilated from obstruction lower down. Rupture is the result of sudden or gradual increase in the back pressure caused by obstruction. Perforation of the pelvis from necrosis due to an impacted calculus is also frequent. An already weakened pelvis may be ruptured by instruments or from increased pressure during employment of the syringe method of pyelography. Perforation has followed the injection of a strong alkali by mistake during pyelography or renal lavage.

WARREN C HUNTER

FIBRINOUS BALLS IN THE URINARY BLADDER F P WEBER, *J Path & Bact* **40** 351, 1935

In a man with a malignant renal tumor the urinary bladder after death contained four fibrinous balls, the largest about the size of a hen's egg. Apparently the fibrin was deposited from the urine, perhaps around fragments from the tumor of the left kidney.

SIMPLE SUPERFICIAL ESOPHAGEAL CAST T C PATTERSON, *J Path & Bact* **40** 559, 1935

Exfoliation of the epidermal lining of the gullet occurs during health, neither infection nor caustic action being causally related. There is usually a history of some earlier mild trauma such as the continued abuse of alcohol, especially strong spirits, the swallowing of hot drinks, the taking of acrid food or the bolting of large mouthfuls. Minor injuries due to foreign bodies are also recorded. Pre-existing cardiospasm and dilatation have sometimes been suspected, largely on the grounds of a history of dysphagia and some apparent obstruction to the passage of food, coupled with the fact that the esophagus had a greater than normal diameter. But this may easily have been the result of stretching of the flimsy tissue during slow spasmodic extrusion. Except in Memmi's case the histories contain little evidence of actual dilatation. In none of the cases is there any indication of syphilitic taint. The patients suffer only moderate and temporary discomfort and practically no shock. The extruded material consists only of the epidermal lining of the gullet, the separation occurring in the subepithelial zone, leaving the deeper parts everywhere intact. The cast is clean, uniformly grayish white, parchment-like and quite free from the foul, discolored, sloughy appearance and deep

penetration seen in corrosive poisoning and phlegmonous esophagitis. Complete and rapid recovery ensues. There is an entire absence of any tendency toward stenosis as a sequela. Very soon after the event the passage appears normal on direct esophagoscopy. New firm epithelium forms *pari passu* with the gradual separation of the cast. In Reichmann's case the forcible application of a metal sound to overcome the obstruction had no ill effect. Le Comte gave emetics with impunity. So slight is the upset that little treatment is necessary beyond judicious resting of the part and temporary restriction to bland fluid nourishment. As a counsel of perfection, rectal feeding has sometimes been carried out for a few days. A peculiar exfoliative tendency seems to be present in certain of the cases. In three cases casts were produced on more than one occasion, and in one of these there was a previous history of severe general eczema. The latter case is possibly analogous to that described by Sligh (1893-1894) in which a man of 36 gave a history of having been taken ill every year since infancy with shedding of the epidermis from the entire surface of the body including the finger-nails and toe-nails. In a few days the exposed new, soft, tender skin became sound and the man could resume work.

FROM THE AUTHOR'S CONCLUSIONS

Microbiology and Parasitology

THE VIRUS OF INGUINAL LYMPHOGRANULOMA J. I. TAMURA, *J. Lab. & Clin. Med.* **20** 393, 1935

When pus from lymphogranuloma inguinale is planted in the medium devised by Martland and his co-workers (*Brit. J. Exper. Path.* **13** 90, 1932) for the cultivation of vaccinia virus the medium becomes cloudy. The agent producing the cloudiness is transmissible in serial cultures, or serial cultures alternating with guinea-pig inoculations. This agent whether in the pus or in the cloudy supernatant culture fluid passes the Berkefeld N filter. The virus is stainable by Hosokawa's eosin-Giemsa method. The heated cultures have been used successfully in making diagnoses by the intradermal skin test and in inducing recoveries through subcutaneous inoculations. Also heated cultures have been used to produce antiserum. Although one can draw no definite conclusions from the few cases in which treatment with the antiserum has been studied the results justify further trial of serum therapy.

FROM THE AUTHOR'S SUMMARY

A NEW VIRUS CAUSING LYMPHOCYTIC MENINGITIS C. ARMSTRONG and J. G. WOOLEY, *Pub. Health Rep.* **50** 537, 1935

The isolation of three similar strains of a newly described virus is reported. Spontaneous infection among stock monkeys has been demonstrated by the isolation of the virus from a noninoculated monkey and by the demonstration of specific antibodies in the serums of 5 of 44 such animals. The possibility that the virus may affect man is suggested since two of the recovered strains are possibly of human origin. The ready and even spontaneous infection of monkeys with the virus, together with the fact that human serums (3 from 166) possessing potent specific antibodies for the virus have been encountered, points in the same direction. As previously noted, the disease in monkeys resembles the human ailment designated as lymphocytic or aseptic meningitis, and serum collected from a person eleven months after a clinical attack of this disease gave strong protection against strains of the experimental virus. The finding of immunity in the serum of an exposed person giving no history suggesting this disease, however, indicates that immunity may develop in the absence of symptoms indicating involvement of the central nervous system.

FROM THE AUTHORS' SUMMARY

SUBFREEZING TEMPERATURES IN PRESERVING MENINGOCOCCI A M PABST, *Pub Health Rep* 50 732, 1935

Ten chosen strains of meningococci have been stored in neutral glycerin at -15°C for two years with no apparent change in viability, morphology or serologic and biochemical characteristics. Two hundred and twenty-three strains have been stored at this temperature on dextrose agar slants both with and without glycerin, with no appreciable loss of viability in the eight months during which they have been under observation.

FROM THE AUTHOR'S SUMMARY

THE GROWTH OF PSITTACOSIS VIRUS IN TISSUE CULTURES J O W BLAND and R G CANTI, *J Path & Bact* 40 231, 1935

The virus of psittacosis was grown in cultures of embryonic chicken tissue. It infects both epithelial cells and fibroblasts. A description is given of the appearance of the intracellular colonies at various stages of their development: (a) in preparations stained by Giemsa's method, (b) in living cultures examined by dark-ground illumination and by transmitted light, and (c) in quick-motion cinematograph films. This study confirms the existence of a developmental cycle in the virus of psittacosis as previously described.

FROM THE AUTHORS' SUMMARY

PSITTACOSIS IN THE DEVELOPING EGG F M BURNET and P M ROUNTREE, *J Path & Bact* 40 471, 1935

Psittacosis virus derived from Australian parrots is readily propagated in the developing egg. Large amounts of virus develop by infection of cells of the ectodermal epithelium, and a characteristic lesion develops. No infection of the embryo proper takes place, and the lesions of the egg membrane are as a rule rapidly resolved after the third or fourth day. The developmental changes in the appearance of the virus bodies described by Bedson and Bland can be well observed in impression preparations from infected egg membrane.

FROM THE AUTHORS' SUMMARY

TUBERCULOUS MENINGITIS IN CHILDREN J W S BLACKLOCK and M A GRIFFIN, *J Path & Bact* 40 489, 1935

Tuberculous meningitis is the most frequent form of meningitis in childhood and accounts for more deaths than all other forms of tuberculosis together. The primary site of infection in a series of 241 patients under 13 years was most frequently thoracic (73.9 per cent), then, in order, abdominal (22.8 per cent), cervical glandular (2.1 per cent) and unknown (1.2 per cent). There was little difference in the distribution according to sex, though meningitis following a primary thoracic lesion was slightly more frequent in girls, and that following primary abdominal tuberculosis was commoner in boys. Of the total number of patients 85.5 per cent were under 6 years of age. The incidence was highest in the late spring and early summer months. The frequent association of tuberculous meningitis and general miliary tuberculosis is discussed, either lesion alone is uncommon. The methods used for isolating and typing tubercle bacilli from the cerebrospinal fluid are described. Human strains were easier to isolate directly by culture than bovine. In one case of infection with a human strain the inoculated guinea-pig gave a negative result whereas the cultures were positive. From a clinical series of cases of cerebral tuberculosis 72 strains were isolated, of which 18.1 per cent were bovine, and from the primary lesions in an autopsy series 114 strains were obtained, of which 24.6 per cent were bovine. The highest percentage of bovine strains occurred in children in the third year of life in both series. The total percentage of bovine strains obtained for meningitis from both the aforementioned

series was 22.5 and for all forms of cerebral tuberculosis 22. This figure is compared with that found by other workers both in this country and abroad. A higher percentage of bovine infections was noted in country than in city children and a probable reason for this is given. Meningitis following primary thoracic lesions was nearly always due to the human type of bacillus, and that following primary abdominal lesions was usually due to the bovine type. From 3 cases of meningitis with the primary lesions verified at autopsy bovine strains were isolated. A case is described in which tubercle bacilli were isolated during life from the cerebrospinal fluid and in which tuberculomas were found post mortem but no meningitis. The bearing of this on the reported recoveries from tuberculous meningitis is discussed.

FROM THE AUTHORS' SUMMARY

THE IMMATURE RABBIT AS AN EXPERIMENTAL URINARY AND FECAL TYPHOID CARRIER. EFFECT OF HEXAMINE TREATMENT. M. COPLAND, J. PATH & BACT 40 521, 1935

By the passage of a laboratory stock culture of *Bacillus typhosus* through a series of rabbits a strain of organisms was isolated which on intravenous injection proved pathogenic in immature rabbits, setting up fecal and urinary carrier conditions in a considerable proportion within the limits of the experiment (sixty-three days). All the twenty-seven controls proved to be infective on postmortem examination. An equal number of immature animals that were similarly inoculated were subsequently treated by oral administration of methenamine and the proportion of carriers, both urinary and fecal, was greatly reduced, no pathogenic organisms being isolated from those which survived the fifteenth to the sixty-third days. Another strain of the organism which after passage was eventually isolated from the feces of a rabbit was utilized for contaminating the green food of immature rabbits which were otherwise being normally fed. In two of ten animals so fed the organisms were excreted in the feces.

FROM THE AUTHOR'S SUMMARY

THE SYPHILITIC VIRUS. W. NYKA, Ann Inst Pasteur 53 243, 1934

The agent of syphilis has two distinct morphologic aspects: the usual spirochete and a filamentous form, between which are a variety of other forms. The transmission of syphilis by the spirochetel form is transitory, the characteristic structural changes of the disease are due to the filamentous form. The spirochetel form is cytotropic and multiplies either in the cytoplasm or in the nucleus, in which the transition to the filamentous form occurs, although the transition occasionally occurs outside of the cell. Filaments multiply by transverse fission. The resulting fragments may develop as spirochetes. Multiplication within the cells destroys the latter. Hepatic cells and fibroblasts are only slightly susceptible, whereas lymphatic cells and nerve cells are quite susceptible. The filamentous form has heretofore been overlooked because of its weak staining properties.

FROM THE AUTHOR'S CONCLUSIONS

MIXED STREPTOCOCCODIPHTHERITIC INFECTION. G. RAMON and M. DJOURICHITCH, Ann Inst Pasteur 53 325, 1934

By using guinea-pigs of standard weight and a virulent strain of the diphtheria bacillus mixed with streptococci or other organisms various data were secured. Certain hemolytic streptococci permitted death of animals with smaller amounts of emulsion of the diphtheria bacillus than killed control animals. Animals with anatoxin (toxoid) uniformly survived mixed injections. Animals given broth with diphtheria bacilli showed no effect, but streptococcus filtrates, as well as killed organisms, increased the mortality. The effect occurred only when mixtures were inoculated at the same site. Serial transfers in animals resulted neither in enhanced

virulence of the mixture nor in virulence or toxigenicity of the diphtheria bacillus used. The authors believe that the action is nonspecific, and that a similar stimulation accounts for the gravity of such mixed infections in man.

M S MARSHALL

MORPHOGENESIS OF NEGRI BODIES S NICOLAU and L KOPCOWSKA, Ann Inst Pasteur **53** 418, 1934

In a previous report (*Compt rend Soc de biol* **115** 262, 1934) the authors considered the dispersion of rabies virus in the nervous system. In the present report (figs 1-22, colored) they consider the development of Negri bodies, typical on the seventh day, in the neurons of the spinal ganglions of rabbits inoculated in the sciatic nerve with street virus. The general morphogenesis is pictured as an orientation and flocculation of Nissl bodies. The authors picture the formation of Negri bodies as a defense reaction on the part of certain cells having exterior contacts, cells not so situated and functionally unable to cope with the virus develop no Negri bodies and are disintegrated.

M S MARSHALL

INCLUSION BODIES IN YELLOW FEVER S NICOLAU, L KOPCOWSKA and M MATHIS, Ann Inst Pasteur **53** 455, 1934

Typical nuclear inclusion bodies were invariably noted in the neurons of the spinal ganglions of monkeys infected by cerebral, intraperitoneal or mosquito-bite injections. A majority of sections from the livers of twenty-two infected human beings also showed characteristic inclusions. In all mice as well as in guinea-pigs dying after subdural inoculation with the yellow fever virus the nerve cells of the neural axis contained inclusion bodies. These bodies were also found, more irregularly, in infected rabbits. The bodies appeared as oxyphilic corpuscles, sometimes surrounded by halos, from 1 to 3 or 4 microns in diameter, with no internal structure, in a rarefied karyoplasm which retained its normal tinctorial properties (color plate, 36 figs).

M S MARSHALL

TISSUE CULTURES IN TYPHUS FEVER A A KRONOWSKI ET AL, Ann Inst Pasteur **53** 654, 1934

Successful efforts were made to grow two laboratory strains of the virus of typhus fever on living tissue. Leukocytes from healthy guinea-pigs were tried also bits of Descemet's membrane from the eye, and a layer of cells from an exudate experimentally produced in the peritoneal cavity of a healthy guinea-pig. Inoculations were made with plasma of the blood of infected guinea-pigs, treated with heparin and rapidly centrifugated. The infective properties of culture originally too weak to infect were demonstrated.

M S MARSHALL

THE PROBLEM OF THE BACTERIAL NUCLEUS C C LINDEGREN, Zentralbl f Bakt (Abt 2) **92** 41, 1935

Referring again to the various kinds of protoplasmic structures suggested as possible for bacteria, it is not possible to have a bacterial cell without a nucleus because that would mean that there were no genes. Life without genes is impossible. A diffuse nucleus is also impossible. The problem of the protoplasmic structure of bacteria therefore resolves itself into that of whether bacterial protoplasm is totally different from that present in any other kind of living form at present known or whether it is possible to demonstrate the presence of nuclear structure within it either by cytologic or by genetic technique.

FROM THE AUTHOR'S SUMMARY

Immunology

HEMOLYTIC ANTIBODIES FOR SHEEP AND OX ERYTHROCYTES IN INFECTIOUS MONONUCLEOSIS G H BAILEY and S RAFFEL, *J Clin Investigation* **14** 228, 1935

Bailey and Raffel found a marked increase of hemolysin for ox red blood cells in three cases of infectious mononucleosis in addition to a high titer of agglutinins and hemolysins for sheep red cells. The agglutinin for ox cells was distinctly elevated only in one of the cases, slightly in the second, and not at all in the third. The antibodies for the sheep as well as for the ox cells were easily removed by boiled and raw red blood cells of the sheep and ox, by those of the latter even more thoroughly than by those of the former. A large variety of other known carriers of the Forssman type of heterophilic antigens failed to absorb the antibodies from the serum of patients with infectious mononucleosis, with the exception of the kidney of the horse and of one strain of *Clostridium Welchii*. But even the latter two antigens did not absorb as regularly and as effectively as did the red cells of the sheep and of the ox. From that, the authors conclude that the antibodies in infectious mononucleosis are not heterophilic, or Forssman, antibodies. They suggest that after a determination of agglutinins for sheep red cells the serum be absorbed with ox red cells boiled. The removal of the antibodies for sheep red cells will indicate the presence of infectious mononucleosis. I DAVIDSON

THE RELATION BETWEEN THE TYPE SPECIFIC CARBOHYDRATES OF PNEUMOCOCCI AND THE BLOOD GROUP SPECIFIC SUBSTANCE A E WITTEBSKY, E NITZ and H SOBOTKA, *J Exper Med* **61** 703, 1935

A relationship between the soluble specific substances of *Pneumococcus* and the blood group substance A of man can be demonstrated by the former's inhibition of sheep cell hemolysis by a group-specific A antiserum. However, there are quantitative differences between the various types. A striking difference exists between the acetyl and the deacetylated polysaccharide of *Pneumococcus* type I, the deacetylated carbohydrate fails to react with the group-specific A antiserum while the acetyl carbohydrate shows a strong reactivity. The minimum amount of the acetyl polysaccharide which inhibits sheep cell hemolysis by A antiserum is almost as small as that of the group-specific carbohydrate isolated by Freudenberg and Eichel from urines of group A. The reactivity of the acetyl polysaccharide can be demonstrated not only by this inhibition of hemolysis test, but also by complement fixation and by inhibition of group-specific iso-agglutination. Fecal filtrates which possess the ability to destroy the blood group specific substances A and B of man also affect the acetyl polysaccharide of *Pneumococcus* type I. After incubation with an effective filtrate of feces the acetyl polysaccharide almost completely loses its potency toward the group-specific A antiserum and also its ability to inhibit the iso-agglutination of A blood cells. The acetyl polysaccharide of *Pneumococcus* type I that has lost its reactivity toward the group-specific A antiserum after treatment with a filtrate of feces still reacts with a type I pneumococcal antiserum that has been previously absorbed with the deacetylated polysaccharide of type I. Thus, the essential effect of a filtrate of feces on the acetyl polysaccharide of type I is not the cleavage of the acetyl group but some other chemical alteration. FROM THE AUTHORS' SUMMARY

THERMOSTABLE BACTERICIDAL SUBSTANCE IN HUMAN SERUM F WULF, *J Immunol* **27** 451, 1934

A thermostable bactericidal substance has been demonstrated to occur in human serum, especially during fever, its effect was particularly seen in tests with meningococci of a strain highly susceptible to the bactericidal substances of serum. The thermostable bactericidal substance was found besides in a few other cases, namely, in tests with two other strains of meningococci, with three strains of

Pfeiffer bacilli, and with a strain of *Diplococcus crassus*—in this case in almost the same frequency as with the highly susceptible strain of meningococci. In tests with meningococci of the highly susceptible strain the thermostable substance was demonstrable in 85 per cent of the febrile patients examined, one-fourth showing a marked action. The thermostable substance could not be demonstrated in 90 per cent of the nonfebrile patients. Injection of sulphur in olive oil seems to stimulate the organism to produce a thermostable bactericidal substance. An increase of the bactericidal substances in active serum was found in tests with a strain of *Diplococcus*, a phenomenon which is probably elicited only under quite special conditions. The thermostable bactericidal substance seems to keep well in vitro. It possesses an enzyme property since it did not become fixed in absorption tests with the strain of meningococci which were killed by it.

FROM THE AUTHOR'S SUMMARY

EXAMINATION OF THE BLOOD QUALITIES M AND N H. ELBEL, *Deutsche Ztschr f d ges gerichtl Med* **24** 242, 1935

One of the great difficulties connected with the preparation of anti-M and anti-N immune serums is the variability of the agglutinogens in the blood cells of different persons whose blood is employed for the absorption of anti-A and anti-B iso-agglutinins from the immune serums prepared in rabbits. Lattes suggested recently the use of boiled red blood cells. The cells are washed, and a fairly thin suspension is prepared and added drop by drop to a boiling physiologic solution of sodium chloride. Elbel tried the use of boiled red blood cells and found that while the agglutinogenic properties were considerably and regularly reduced the disadvantage was offset by a number of advantages: (a) A batch of blood with the same agglutinogenic properties can be kept for a long period, (b) the blood cells remaining from specimens used for the Wassermann test can be sterilized and used, (c) the absorbed serum is not discolored.

I. DAVIDSOHN

HETEROPHILIC ANTIBODIES IN GLANDULAR FEVER (INFECTIOUS MONONUCLEOSIS)

L. MEIJLER and R. J. SIEMELINK, *Nederl tijdschr v geneesk* **28** 1952, 1934

The test for heterophilic antibodies described by Paul and Bunnell established the diagnosis of infectious mononucleosis in 5 cases. A sixth case, in which the titer was 1:16 (by the first method of Davidsohn) is included by Meijler and Siemelink, although they assume that, to be diagnostic, the titer has to be 1:32. The first case, in the early course, had to be differentiated from appendicitis and later from acute leukemia. A rash and hemorrhages in the skin were also notable features. Another case was marked by abdominal findings and diarrhea. The serums of 400 patients with various diseases were studied as controls. The highest titer was 1:8 if one excepts the titers of 4 patients who had received injections of horse serum and 2 patients (1 with jaundice and 1 with a subacute leukemic myelosis) whose elevated titers of heterophilic antibodies could not be explained.

I. DAVIDSOHN

Tumors

CARCINOMATOUS ENDARTERITIS OF PULMONARY VESSELS RESULTING IN FAILURE OF RIGHT VENTRICLE EDWARD B. GREENSPAN, *Arch Int Med* **54** 625, 1934

Four cases of carcinoma in the lymph vessels of the lung are reported, in three of which the condition was secondary to scirrhus carcinoma of the stomach, and in one, secondary to adenocarcinoma of the sigmoid. The four patients presented symptoms of cough, tachypnea and cyanosis with inconspicuous physical signs. The cases in which the involvement of the pulmonary lymph vessels was secondary to gastric carcinoma presented diffuse obliterative endarteritis of many

pulmonary arterioles and small arteries, due chiefly to the influence of the carcinomatous growth in the neighboring perivascular lymphatics, rarely to carcinomatous emboli. In two of the cases, failure of the right ventricle of the heart was the direct result of the diffuse obliterative changes in the pulmonary vessels. In cases of right ventricular cardiac failure presenting no significant pulmonary or cardiac findings, the possibility of a diffuse secondary carcinoma in the lymph vessels of the lungs with accompanying obliterative endarteritis should be considered.

FROM THE AUTHOR'S SUMMARY

A VIRUS-INDUCED MAMMALIAN GROWTH WITH THE CHARACTERS OF A TUMOR (THE SHOPE RABBIT PAPILLOMA) P. ROUS and J. W. BRAD, *J. Exper. Med.* 60: 701, 723 and 741, 1934

Growth on Implantation Within Favorable Hosts—Rabbit papillomas developing on the skin as the result of virus inoculations can be readily transferred to the inner organs of favorable hosts by implanting bits of the living tissue. The growths thus produced proliferate actively, as a rule, and frequently cause death. Often they are markedly invasive and destructive, and they tend to recur after excision. Bacterial infection may greatly enhance their malignancy. Accidental dissemination may occur during operation, and distribution to the peritoneal surface has been repeatedly noted. There may be no cellular reaction about the invading epithelium of interior growths, but usually some new formation of connective tissue takes place, its amount varying inversely with the rate of epithelial proliferation. An immediate reason for the inflammatory changes and scarring found beneath long-established skin papillomas exists in the trauma and secondary infection to which the projecting, necrotizing masses have been subjected. In animals dying of progressively enlarging interior growths the skin papillomas may long have been stationary in size. The growths appearing after the transfer of papillomatous tissue to the inner organs are due to the survival and multiplication of the transplanted cells. However, the virus can be readily recovered from them, in the case of wild rabbits. No distinctive changes in the blood of the host have been found. The virus itself is highly specific for the epithelium of the skin, failing to act not only on that of the other organs thus far tested but even on embryonic skin. The papilloma frequently penetrates into the blood and lymph vessels, especially at the edge of an implantation growth. The intravascular injection of fragments of it sometimes results in pulmonary nodules of characteristic morphology. These are due to survival and proliferation of the injected cells. Secondary nodules have been encountered at autopsy in a lymph gland and in the lungs, but under conditions more suggestive of operative dissemination of the growth than of true metastasis. Implantation growths of the papilloma in favorable hosts have the morphology of epidermoid tumors of greater or less malignancy. They behave as these do and elicit similar changes in the surrounding tissue.

Experimental Alterations of the Growth on the Skin—The injection of scarlet red into the skin about rabbit papillomas resulting from virus inoculations causes them to invade the underlying tissue and form large fleshy masses beneath the surface. Histologically these appear malignant, and they frequently invade the blood vessels. Covering young papillomas with a layer of collodion causes them to burrow downward, which results in discoid masses that enlarge progressively by expansive growth beneath the epidermis and by invasion. Such masses, like the nodules resulting from implantation, have the papillae turned toward their interior, the apparent reverse of what occurs when the growth is situated on the skin surface. The reasons for this are analyzed. The peculiarities of the host influence skin papillomas not a little, as is plain from the forms they assume, but the epithelial changes induced by the virus take a single direction, and no significant variations from type have been encountered. Local or generalized retrogression of the experimentally induced papilloma is not uncommon. The histologic alterations that take place are identical with those attending retrogression.

of the epidermoid tumors and the reactive changes taking place in the surrounding tissue are also like those about such tumors. The slowing and cessation of growth that occur secondarily in virus-induced skin papillomas are associated with the formation under them of a dense layer of connective tissue, and to this their behavior is attributable. Similar findings have often been recorded for tumors, notably for the epidermoid cancers produced in rabbits by tarring.

Further Characters of the Growth, General Discussion—Experimental study of the rabbit papilloma of Shope, a growth caused by a virus, has shown that it possesses the immediate characters whereby tumors are recognized. Often it looks and acts like a malignant neoplasm. It differs from the tumors as a group, however, in its incidence, which is that of an infectious process, and from other mammalian tumors in that its cause has been demonstrated. The possible bearing of the findings on the problem of tumor causation is discussed. The morphology and behavior of the generality of tumors can no longer be taken to exclude the possibility that these are produced by extraneous, living entities. The incidence of some of the tumors, at least, and the failure to demonstrate their cause can both be explained on the assumption that they are due to such entities widely distributed in or on the animal population but effective only under special circumstances. Present knowledge makes this assumption reasonable as a basis for further work.

FROM THE AUTHORS' SUMMARIES

GELATINOUS CARCINOMA OF THE BREAST B. J. LEE, H. HAUSER and G. T. PACK,
Surg., Gynec. & Obst. **59** 841, 1934

Gelatinous (colloid) carcinoma occurs in many organs which normally secrete mucus. Of the cancers of the breast this form constitutes from 1 to 2 per cent. Two forms are recognized: (a) the primary gelatinous form in which the gelatinous features predominate and (b) ordinary carcinoma with secondary gelatinous degeneration. The latter may be termed myxoid or mucoid carcinoma depending on whether the gelatinous changes arise by metaplasia in the connective tissue or by secretion directly from the carcinoma cells. Of the two origins for the gelatinous material, the latter is the more common. This form of carcinoma is usually slower in growth than ordinary carcinoma, and one of the reasons is that the tumor often arises on the basis of a preexisting benign mammary adenoma. In the thirty cases studied no essential differences were observed in age, sex, race distribution, history of lactation and trauma between gelatinous carcinoma and ordinary carcinoma. Metastases occur comparatively late, are commonly confined to the axillary nodes and do not necessarily show gelatinous changes. The end-results of the writers' cases indicate an appreciably higher percentage of cures than with other forms of cancer of the breast.

FROM THE AUTHORS' SUMMARY (WARREN C. HUNTER)

EXPERIMENTAL BONE SARCOMA A. BRUNSCHWIG and P. H. HARMON, Surg.,
Gynec. & Obst. **60** 30, 1935

A transplantable rat sarcoma originating in the abdominal wall, the cells of which do not exhibit osteogenic properties, was inoculated into the medullary cavity and beneath the periosteum of long bones. The tumor penetrated the cortex, elevated the periosteum and caused it to lay down new bone in the form of radiating trabeculae within the tumor. Evidence is presented to show that all of the new bone in the tumor was periosteal in origin and not the result of osteoblastic properties acquired by the tumor cells from implantation within bone. In man elevation of the periosteum by osteogenic sarcoma may be an important contributing factor to the formation of new bone even though the tumor cells themselves have osteogenic properties. The mode of new bone formation in Ewing's sarcoma is quite analogous to that in the experimental tumors described here.

FROM THE AUTHORS' SUMMARY (WARREN C. HUNTER)

GROWTH-PROMOTING AND GROWTH-INHIBITING PROPERTIES IN BLOOD OF MICE
RESISTANT TO A TAR-SARCOMA F C PARUS and E W MILLER, Brit J
Exper Path **15** 207, 1934

A difference has been found to exist between mice naturally immune to tar sarcoma NT₂ and mice resistant to this tumor as regards certain properties of their serum and plasma. When the immune mice have received three inoculations of NT₂ their serum and plasma do not inhibit the growth of this tumor in vitro but, on the contrary, definitely stimulate it, the serum and plasma of the resistant mice do not always have this stimulating effect but may occasionally inhibit the growth slightly. A similar difference has been found to exist between immune and resistant mice which have received eight inoculations each, but it is less definite than after three inoculations. The serum from resistant mice which have received three inoculations of NT₂ definitely stimulates the growth of c63 in vitro, and slightly stimulates the growth of normal mouse heart. The serum from immune mice which have received three inoculations does not affect the growth in vitro of normal mouse heart. No adverse effect on the growth of NT₂ in vitro is shown by the serum or plasma of mice which have once been inoculated with NT₂ and in which the tumors are actively growing, or are regressing, or have completely regressed. In the first two cases growth is, if anything, slightly stimulated. Certain objections to the present method of investigation are discussed, and comparisons are drawn between conditions in vivo and in vitro.

FROM THE AUTHORS' SUMMARY

INJURY IN THE GENESIS OF TUMOURS OF THE GONADS R A WILLIS, Brit J
Exper Path **15** 234, 1934

In the experiments recorded, 107 rats (82 males and 25 females) were used. In the different groups the animals were of different ages from 2 months up to middle adult age. The testes and ovaries were subjected to a great variety of traumatic and chemical injuries, and the animals were kept for from twenty-five to thirty-five weeks thereafter. The changes observed in the damaged organs were those of necrosis and its sequelae, no tumors appeared. These experiments, then, so far as they go, afford no evidence that local injury is capable of evoking neoplasia in gonadal tissue. While a larger series of experiments on a variety of mammals is desirable, the result obtained in this investigation at least serves to strengthen the suspicion that injury and inflammatory processes play no more than a coincidental part in the histories of testicular or ovarian tumors.

FROM THE AUTHOR'S SUMMARY

CANCER OF SKIN AND INCREASE IN INCIDENCE OF PRIMARY TUMOURS OF LUNG
IN MICE EXPOSED TO DUST OBTAINED FROM TARRIED ROADS J A CAMPBELL,
Brit J Exper Path **15** 287, 1934

Mice were repeatedly exposed to dust containing about 2 per cent tar obtained by sweepings from tarred roads. Cancer of the skin developed in 70 per cent of those surviving long enough. The incidence of primary adenoma of the lung was increased to ten times that of the controls, the lungs of the dusted mice contained much dust. The breathing of carbon monoxide, if anything, retarded the effects of dusting. The bearing of this research on the debated increase in tumors of the human lung cannot be assessed at present. The mice were exposed to much more excessive dusting than occurs with man. Cleanliness prevents cancer of the skin in man, and the natural mechanism for removal of dusts from the healthy lung may suffice for the small amounts of dust inhaled. There is the further question whether these tumors of mice may be compared with those of the human lung. The experiment with mice is to be repeated, and attempts will be made to transplant some of the tumors, the effects of dust, with the tar products removed, will also be studied.

FROM THE AUTHOR'S SUMMARY

THE PRODUCTION OF TUMOURS IN FOWL WITH A COLLOIDAL SOLUTION OF 1 2
5 6-DIBENZANTHRACENE I BERENBLUM and L P KENDAL, Brit J Exper
Path **15** 366, 1934

Repeated injections of a 0.007 per cent colloidal solution of dibenzanthracene in water into the breast muscle led to the production of spindle-cell sarcoma in eight of twelve birds which survived more than twelve weeks. The total amount of dibenzanthracene injected was about 18 mg. Secondary deposits were found in three birds. Five of these tumors were transplanted into other birds, and two of them grew successfully. In one of these inoculated birds metastases were present in the lungs. The administration of kieselguhr together with colloidal dibenzanthracene did not appear to influence significantly the development of the tumors. Eighteen months after intramuscular injections of 0.6 mg of dibenzanthracene, none of the substance could be detected in the muscle about the site of injection.

AUTHORS' SUMMARY

THE STRUCTURE OF TERATOMA R A WILLIS, J Path & Bact **40** 1, 1935

Fourteen teratomas have been studied and mapped in serial slabs and the distribution and relationships of the component tissues have been worked out. In the identification of many of the tissues difficulties are encountered. An intimate knowledge of the histology of adult and embryonic tissues is necessary. Immature glandular and neuro-epithelial tissues are readily confused with one another. For the clear delineation of neuroglial tissue and especially for the identification of immature neuroglial cells Cajal's gold method is desirable. Unless accompanied by hair follicles or cutaneous glands, stratified squamous epithelium cannot be identified as epidermal, since squamous metaplasia frequently occurs in the glandular components of teratoma. Renal tissue is a rare component, in the identification of which special caution is essential, it may be immature even in an otherwise fully differentiated growth. Function in teratomatous tissues—secretion, hematopoiesis, movement, nervous activity—is of interest in connection with the problems of the prefunctional and functional differentiation of tissues. Teratomas of the testis are almost always malignant, and with rare exceptions the malignancy involves many or all of the component tissues, though perhaps in different degrees. Most teratomas exhibit no signs of somatic axiation, segmentation or delamination of germ layers, they possess no organs or true somatic regions, they exhibit anomalous excess of some components and anomalous absence of others, and they exhibit abnormal mixtures and relationships of tissues and coexistence of tissues of widely different degrees of maturity. For these reasons, the view that a teratoma is homologous with a fetus must be rejected, this means rejection of the hypotheses that the teratoma represents a twin inclusion and that it represents parthenogenesis. It is noteworthy that on incomplete examination a teratoma may present a spurious resemblance to a fetus. Tissue correlations, probably similar to those obtaining in normal ontogeny, are evident in teratomas. Various growing epithelia appear to induce specific changes in associated plastic mesenchyme, certain glandular epithelia evoke the formation of smooth muscle, young central nervous tissue evokes chondrification, tooth development exhibits its characteristic and complex tissue correlations, respiratory mucosa induces the formation of cartilage, nervous tissue the formation of a meninx-like sheath or of nerve-sheath elements, epidermis the formation of dermis and certain mucosal epithelia the formation of lymphoid tissue. Teratoma constitutes a field of study in which pathologist and embryologist have a common interest and can mutually benefit each other.

FROM THE AUTHOR'S SUMMARY

LEUKEMIA TRANSMISSIBLE BY A SPINDLE-CELL SARCOMA IN THE MOUSE L D
PARSONS, J Path & Bact **40** 45, 1935

A leukemic condition in mice coincident with and transmissible by the grafting of a spindle-cell sarcoma is described. Examination of the cells of the blood of the affected animals shows the leukemia to be myeloid, with large numbers of

pathologic polymorphonuclears The hemohistioblast, also present in the blood stream in this condition, is suggested as the primitive cell giving rise directly to pathologic polymorphonuclears of the granular series The activity of the fixed cells of the spleen, liver and other organs appears to be responsible for the gradual appearance and rise in number of primitive cells in the blood stream

FROM THE AUTHOR'S SUMMARY

CARCINOMA OF THE INTESTINE IN RATS R A WILLIS, J Path & Bact **40** 187, 1935

The appearance of two almost identical carcinomas in two closely related young rats of the same age gives food for thought Suppose that the experiments performed on these rats had been dietetic, say the administration of some carcinogenic substance with the food Very erroneous conclusions could easily have been reached This report clearly emphasizes the need for great caution in making deductions concerning the incidence or the genesis of neoplasms in experimental animals, unless adequate numbers and full controls are used

FROM THE AUTHOR'S COMMENT

THE ACTION OF RADIUM ON THE INORGANIC STRUCTURE OF TUMOUR CELLS AS SHOWN BY MICROINCINERATION E S HORNING, Scient Rep Invest Imp Cancer Research Fund **11** 67, 1934

The inorganic structure of the cells of adenocarcinoma 27 following microincineration is briefly described and compared in detail with that in similar incinerated sections of the tumor irradiated and examined after periods ranging from six hours to twenty-four days An increased cytoplasmic ash is recorded in the tumor cells from six to eight hours after irradiation, which at this phase is apparently not the result of immediate degenerative changes This phenomenon is maintained in the cytoplasm twenty-four hours after irradiation, and is accompanied by cellular hypertrophy, which is more marked on the third day The maximum increase of mineral salts occurs on the sixth day following irradiation, at which time degenerative areas are found in all tumor cell masses These phenomena may be partly due to the secondary effect of irradiation on the vascular supply The degeneration may be followed clearly in the inorganic residues of the tumor cells owing to the absence of ash in the fat globules which collect in the cytoplasm The hypertrophied nucleoli and increased chromatin masses are clearly recognizable in the ash The multinucleate cell formation and final disintegration stages in the tumor cells also involve characteristic alterations in the inorganic material

FROM THE AUTHOR'S SUMMARY

THE ACTION OF RADIUM ON CANCER CELLS H G CRABTREE and W CRAMER, Scient Rep Invest Imp Cancer Research Fund **11** 75, 89 and 103, 1934

Effects of Hydrocyanic Acid, Iodo-Acetic Acid and Sodium Fluoride on the Metabolism and Transplantability of Cancer Cells—Hydrocyanic acid produces primarily an inhibition of respiration which is of the order of from 85 to 95 per cent in concentrations of from one-thousandth to five-hundredth molar As a secondary effect of this inhibition the aerobic glycolysis of tumor tissue increases to a value approaching that of anaerobic glycolysis The effect of hydrocyanic acid is completely reversible in all concentrations up to twentieth molar The primary effect of sodium fluoride and iodo-acetic acid is an inhibition of aerobic glycolysis, which increases progressively with increasing concentration This direct checking of aerobic glycolysis is accompanied by a small indirect inhibition of respiration which increases with the time of exposure Within certain characteristic concentrations these inhibitions are reversible after an experimental period of one hour If these limits of concentration are exceeded, these two substances produce an irreversible damage in tumor cells This damaging action appears even with

concentrations which are insufficient to effect a complete or almost complete inhibition of glycolysis. There is a close parallelism between the degree of metabolic recovery and the effect on the viability of the cell as measured by subsequent transplantations.

Some Factors Determining the Susceptibility of Cancer Cells to Radium—It is possible to produce experimentally great variations in the susceptibility of cancer cells to radium by acting on the respiratory mechanism of the cell. It is not possible to do so by acting on the glycolytic mechanism. The measures used for action on the respiratory mechanism were anaerobiosis, subjection to hydrocyanic acid and subjection to cold. Although all three have the same general action of diminishing the functional activity of the respiratory mechanism, their effects on the susceptibility to radium are in opposite directions. Anaerobiosis diminishes, hydrocyanic acid and cold increase the susceptibility to radium.

Factors Determining the Susceptibility of Cancer Cells to Gamma Radiation—Pure gamma rays have the same biologic effect on cancer cells in vitro as a mixture of beta and gamma rays. In both cases the functional condition of the respiratory system determines the biologic response of the cell, while the glycolytic mechanism is not primarily concerned.

FROM THE AUTHORS' SUMMARIES

VARIATIONS OF METABOLISM AND RADIO-SENSITIVITY OF TISSUES IN BICARBONATE- AND PHOSPHATE-BUFFERED MEDIA H. G. CRABTREE, *Scient Rep Invest Imp Cancer Research Fund* **11** 119, 1934

A comparison has been made of the carbohydrate metabolism exhibited in vitro by tissues suspended in saline mediums buffered with phosphate and bicarbonate, respectively. The respiration of rat liver and Jensen's rat sarcoma was well maintained during four hours in both mediums, but the values found in phosphate-Ringer solution were consistently from 20 to 30 per cent lower than those in bicarbonate-Ringer solution. The susceptibility of tumor tissues to gamma radiation has been shown to be a function of the condition of the respiratory system. When tumor slices are suspended in bicarbonate-buffered and phosphate-buffered mediums, respectively, treated with gamma radiation and subsequently transplanted, the damaging influence of phosphate-Ringer solution is made apparent by the lower number of "takes" or the subsequent slower rate of growth of the transplants. This indirect method of showing the adverse effect of phosphate-Ringer solution confirms the results obtained by direct measurement of carbohydrate metabolism. Phosphate-Ringer solution, in some unknown manner, damages the respiratory system of tumor tissues.

FROM THE AUTHOR'S SUMMARY

THE THERAPEUTIC ACTION OF RADIUM ON SPONTANEOUS MAMMARY CARCINOMA OF THE MOUSE W. CRAMER, *Scient Rep Invest Imp Cancer Research Fund* **11** 127, 1934

The spontaneous mammary carcinomas of the mouse show differences in sensitivity to radium. They present, therefore, a suitable material for the study of the factors which determine the radiosensitivity and radioresistance of malignant new growths. With a given dose which in the radiosensitive tumor produces rapid regression, the radioresistant tumor shows only an arrest of growth or a partial regression. In both cases the action is only a local one and is restricted to the irradiated area. With the doses given, no evidence has been obtained of a stimulation of growth or of an enhanced metastatic dissemination. The process of regression in the radiosensitive tumors corresponds closely to that described for a radiosensitive transplantable tumor of the mouse. Histologically it begins with an invasion of the tumor by macrophages. This leads to the formation of a massive stroma overgrowing the tumor and splitting the malignant parenchyma into narrow strands of living and viable cells. It is pointed out that, although

histologically these irradiated viable cells appear normal, they have undergone a change so far as they have been temporarily deprived of their power of growth. The process of regression as a whole is, as previously shown, not due to a direct lethal action on the cancer cells nor entirely to an action on the tumor bed, but is the result of a damage inflicted on both the tumor parenchyma and the tumor bed, followed by repair. In the radioresistant tumors irradiation does not produce a macrophage invasion and a subsequent overgrowth of the stroma. It is shown experimentally that the temporary loss of growth which malignant cells undergo after irradiation is due to a direct action on these cells. They may remain in this dormant condition over a relatively long period, but eventually they recover from it and resume their usual rate of growth. The bearing of this phenomenon on the clinical results of radiotherapy is discussed, and a distinction is drawn between the true recurrences due to this condition and the apparent recurrences which are really the result of a new development of malignancy due to an insufficiently extensive irradiation. The fact that it is possible to bring about locally the disappearance of a malignant new growth by an agent which does not kill all the malignant cells directly, but only inflicts on them a temporary damage of a specific nature, indicates the possibility of a systemic therapy of cancer along the same lines.

FROM THE AUTHOR'S SUMMARY

Technical

"SPOT" PREPARATIONS IN THE POSTMORTEM DIAGNOSIS OF HEMATOPOIETIC DISEASES. A. F. ZANATI, *Virchows Arch f path Anat* **293** 335, 1934

"Spot" preparations made at the time of autopsy from the various organs and tissues are highly recommended as an aid in the diagnosis of diseases of the hematopoietic organs. They may be stained by a variety of methods and yield more satisfactory results than smear or "fluck drop" preparations. O. T. SCHULTZ

HISTOLOGIC DEMONSTRATION OF GLYCOGEN IN MUSCLE. A. NOLL, *Virchows Arch f path Anat* **293** 409, 1935

Pieces of fresh muscle from 0.5 to 1 cm long and a few millimeters wide are placed in 5 per cent aqueous potassium hydroxide at room temperature for a maximum of four hours. The tissue is then washed in water for one minute. It is then placed in 96 per cent alcohol, which is changed several times during the first few hours, followed by immersion in absolute alcohol, in which it remains for at least twenty-four hours. It is embedded in pyroxylin (celloidin). The sections may be stained by Best's carmine method or with iodine. O. T. SCHULTZ

PRESERVATION OF SHEEP ERYTHROCYTES FOR COMPLEMENT FIXATION. S. GINSBURG and R. SELIKOWA, *Ztschr f Immunitätsforsch u exper Therap* **83** 157, 1934

The sheep blood was defibrinated and washed twice with a modified Ringer-Locke solution (the dextrose is left out and boric acid crystals are added in the concentration of 1 per cent). The cells are suspended in the same solution in the proportion of 1:33. The red cells remained satisfactory for hemolytic and complement-fixation tests for from ten to twenty days, their fragility remained unchanged for a similar period. The modified Ringer-Locke solution (without the boric acid) is sterilized in the Arnold sterilizer for thirty minutes. A precipitate forms during sterilization which has no harmful effect. The boric acid crystals are now added. The solution keeps well for from one to two months at room temperature.

I. DAVIDSOHN

FLOCCULATION TEST FOR SYPHILIS WITH THE ANTIGEN ABF (ANTIGENE-BRUXELLES-FLOCCULATION) M STERN, *Ztschr f Immunitätsforsch u exper Therap* **83** 228, 1934

The technic of the preparation of the antigen, which was originally suggested by Bordet, is not given. The method of its dilution and the technic of the test are described and impress one by their simplicity. The results were compared with those of the Wassermann test (Wadsworth antigen), the micro test, the clearing test of Meinicke and the Kahn test. The test proved highly specific with comparatively few false reactions. The antigen can be used for the test with cerebrospinal fluid, but a large amount of the fluid is needed. I DAVIDSOHN

PRESERVATION OF COMPLEMENT H MIRDAMADI and K GIESF, *Ztschr f Immunitätsforsch u exper Therap* **83** 304, 1934

Complement was diluted with an equal volume of a 12 per cent solution of sodium acetate containing 4 per cent boric acid. The serum of the guinea-pigs which contained normal antisheep lysin was eliminated. The preserved complement kept well for a number of weeks. I DAVIDSOHN

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, Feb 28, 1935

WILLIAM C VON GLAHN, *President, in the Chair*

IRVING GRAEF, *Secretary*

ABSCESS OF THE LIVER DUE TO FRIEDLANDER'S BACILLUS CHARLES T OLCOTT

The literature concerning abscesses of the liver caused by Friedlander's bacillus has been reviewed. Five cases have been found in which there were pulmonic and hepatic lesions, and five others in which the lungs were not involved (Gilbert-Dreyfuss and Dausse, *C Rev med-chir d mal du foie* 4 481, 1929 [references] Lutembacher, R, and Debais *Ann de med* 8 460, 1920 Le Sourd and Draillard *Gaz d hôp* 105 1185, 1932). In the latter group the kidney was also involved three times, the meninges and spleen each twice and the gallbladder once.

The case presented was that of a woman 51 years of age on the service of Dr E F DuBois, New York Hospital. She had had diabetes mellitus for eleven years and infection of one foot necessitating amputation three years before admission to the hospital. She had had a nonproductive cough and repeated vomiting on admission. The abdomen was diffusely tender, especially in the right upper quadrant, and there was a palpable mass extending 8 cm below the costal border. The heart was enlarged, and there were some râles in the chest. Blood cultures showed Friedlander's bacillus. The white blood counts showed 44,000 and 23,200 white cells with 90 per cent polymorphonuclears, two-thirds immature forms. The patient died two days after admission.

At autopsy (Henry S Dunning) there was advanced arteriosclerosis with fibrosis of the pancreas. The heart was dilated and hypertrophied. The lungs showed congestion and edema but no pneumonia. The serous cavities were clear. The liver weighed 2,800 Gm, and there was a multilocular abscess cavity 11 cm across in the right lobe. This contained thick yellow liquid. The edges were irregularly trabeculated and white. There was surrounding congestion. Microscopically, polymorphonuclear cells were found in the central necrotic area. These were surrounded by mononuclear cells and fibroblasts. The spleen was soft and enlarged, and it showed infarcts, one of which was filled with pus.

Smears from the liver and spleen showed gram-negative encapsulated bacilli. Cultures from the cardiac blood, spleen and liver showed Friedlander's bacillus.

ATROPHY OF THE CORTEX OF THE ADRENAL GLAND WITH ADDISON'S DISEASE JAMES A MOORE (by invitation)

A 26 year old white married woman was admitted to the New York Hospital because of general weakness and fatigue of two months' duration. The family and past histories were essentially negative. For the past two months prior to her entry into the hospital there were increasing weakness and fatigue, loss of weight, nausea, vomiting and increasing brownish pigmentation of the skin.

Physical examination showed moderate dehydration, apparent loss of weight and increased brownish pigmentation of the skin over the flanks, abdomen, arm pits, nipples and buccal mucosa. The apex impulse was weak and diffuse but within the normal boundaries. The blood pressure was 74 systolic and 46 diastolic. The urine was essentially normal. The red blood cell count and the hemoglobin con-

tent were normal. The white blood cell count was 15,400, with 54 per cent polymorphonuclear leukocytes. The Kline test was negative. The urea nitrogen was 44 mg per hundred cubic centimeters of blood, and the plasma chlorides 600 mg. The patient declined steadily and died three days after admission without apparent response to the administration of large amounts of sodium chloride by vein or to the intravenous use of 20 cc of extract of adrenal cortex.

The essential features of the autopsy (Henry S. Dunning) were as follows. The skin and buccal mucosa were pigmented light brown, as described. The heart weighed 205 Gm. The circumference of the abdominal portion of the aorta was 25 cm. The coronary, splenic and renal arteries had unusually thin walls. There was a small partially calcified fibrocaseous nodule in the upper lobe of the right lung with a similar nodule in the tracheobronchial lymph nodes draining that area. The thymus gland weighed 75 Gm and was grossly normal. Grossly the thyroid gland, pituitary gland, liver, pancreas, biliary tract and spleen were not remarkable. The right adrenal gland was gray-brown, homogeneous, soft and friable. It measured 2.5 cm in length, 2 cm in width and 0.3 cm in thickness, with no differentiation into cortex and medulla. The left adrenal gland measured 4.5 cm in length, 1.9 cm in width and 7 mm in thickness, with definite differentiation into cortex and medulla. The cortex was gray-brown, homogeneous, fairly firm, with a slightly lobulated surface, and measured 0.5 mm in thickness. The medulla was gray, soft and friable. At the lower pole of this gland there was a small subcapsular hemorrhage. The kidneys showed some brown pigmentation of the pyramids. The ureters, bladder and genital organs were normal.

On microscopic examination the basal cells of the pigmented skin and buccal mucosa were filled with fine brown pigment granules. The rectus muscle fibers showed some Zenker's hyaline degeneration. The thymus gland was not remarkable. The thyroid gland showed diffuse infiltration with lymphocytes and plasma cells, and the acini were lined by unusually tall epithelial cells and for the most part were devoid of colloid. The pituitary gland was normal. The capsule of the left adrenal gland was of normal thickness. The area between the medulla and the capsule was much reduced and showed only a few isolated islands of what appeared to be cortical cells with irregular outline, pink-staining cytoplasm and hyperchromatic vesicular nuclei, surrounded by congested capillaries, remaining stroma and a dense lymphoid infiltration. The medulla showed considerable lymphoid infiltration with some degeneration of the chromaffin cells in these areas. A section from the lower part of the right adrenal gland consisted entirely of well preserved medullary tissue with a few focal accumulations of lymphocytes. This section showed neither cortex nor capsule. Stains for acid-fast bacilli were negative.

This case conforms closely with those reported by Brenner and others. Both adrenal glands showed advanced atrophy of the cortex. The medulla in each instance was essentially normal in appearance except for the lymphoid infiltration. There were associated changes in the thyroid gland and rectus muscle fibers similar to those described in other case reports.

DISCUSSION

WILLIAM C. VON GLAHN. Since 1926 my associates and I have had five cases of Addison's disease following atrophy of the adrenal whereas in the past twenty-four years only six cases due to tuberculosis of the adrenal were observed. I am wondering if this is the experience of other pathologists?

ALFRED PLAUT. In the last five years I have seen two cases of Addison's disease, one associated with tuberculosis and one with atrophy.

ROBERT A. MOORE. It seems to me that cases of this type present certain problems and also certain opportunities for the study of the physiology of the adrenals. At New York Hospital in the past two years we have had two cases of Addison's disease, one was tuberculous and the other was this case of atrophy. I do not know what the relative importance of these associated conditions is, but

in the large series of 500 cases it is probable that a true index of the relative amount of atrophy is given, namely, 10 per cent. There is some indication, as Wells has pointed out in reviewing his cases, that this type of change is not dissimilar from acute yellow atrophy in which there is a profound degeneration of the parenchyma without fibroblastic proliferation.

One other point in connection with the morphology of the disease. The thymus was not enlarged, but certainly there were hypoplastic changes in the vessels of this patient. There was lymphoid infiltration in the thyroid, and the lymphatic tissue in the body was increased over that which would be expected in a person of this age. That brings up again the possible relationship of the adrenal to the so-called status thymicolymphaticus.

OSTEOID OSTEOMA HENRY L. JAFFE

(Material was presented on a bone lesion designated "osteoid osteoma") Osteoid osteoma appears to be a distinctive benign bone tumor. It arises from osteoblasts. Irregularly between the osteoblasts intercellular material develops. In this way patches of osteoid tissue are formed. In the further progress of the lesion the osteoid becomes calcified and even converted into atypical bone. In the course of the conversion osteoclasts appear. Sometimes the lesions are rather vascular.

Osteoid osteoma is by no means a rare condition. Seven instances have been noted and studied in the laboratory during eighteen months. Nevertheless, this lesion has been mentioned in the literature only three times and has never before been interpreted as a distinctive bone tumor. Heine, in 1927, described one instance of the lesion under the title "A Bone Sequestrum in Process of Being Reincorporated into the Basal Phalanx of the Ring Finger." Hitzrot, in 1929, under the title "Sclerosing Osteomyelitis of the Carpal Scaphoid," presented before the New York Surgical Society a case the roentgenogram of which published in the transactions of the society closely resembles the roentgenogram in some of our cases. Bergstrand, in 1930, under the title "A Peculiar and Probably Not Hitherto Described Osteoblastic Disease in the Long Bones of the Hand and Foot," reported two cases that seem quite clearly to be instances of what I have called osteoid osteoma. He was at a loss to classify the condition but held that it was neither a tumor nor an inflammatory process, regarding it as a reactivation of an embryonal rest.

All the cases which I have observed had the following features in common. The patients were adolescents or young adults. The principal complaint was local pain. Uniformly the lesion originated in spongy bone areas. As observed roentgenographically the pathologic areas were roundish and clearly circumscribed. The lesions were small and closely similar in size. In every case operation was performed on the assumption that the lesion was an inflammatory one. Complete eradication resulted in the eventual disappearance of all symptoms without recurrence of the local condition.

It was indicated that osteoid osteoma is possibly related to the metacarpal bone tumor previously described by Dr. Mayer and Dr. Jaffe under the title "An Osteoblastic, Osteoid-Tissue-Forming Tumor of a Metacarpal Bone." This metacarpal bone tumor is possibly an osteoid osteoma which, because of incomplete removal, continued to proliferate and began to take on the characteristics of osteogenic sarcoma.

As to differential diagnosis, osteoid osteoma has no features suggesting that it has an inflammatory origin or an origin from an embryonic rest, or that it represents an unfamiliar healing stage of a giant cell tumor, localized osteitis fibrosa or cyst. Altogether, it seems fair to assume that osteoid osteoma is a distinctive benign osteoblastic bone tumor which has hitherto been overlooked as such, and the true incidence of which is still to be determined.

It is suggested that osteoid osteoma is possibly the benign counterpart of malignant osteogenic sarcoma

This article will be published in full in the *Archives of Surgery*

HISTOGENESIS OF LYMPHOSARCOMATOSIS J C EHRLICH (by invitation) and I E GERBER

Histologic studies of the biopsy and necropsy material from eighteen cases of lymphosarcomatosis revealed varied histologic pictures which could be grouped into three main types on the basis of the morphologic characteristics of the predominating cell in each case. There were found, first, cases in which large pale cells in either symplasmic or reticular arrangement predominated. Then there was a group in which the lymphosarcomatous tissues were composed of mixed cells, partly reticular, as in the former group, and partly free. The morphology of these free cells resembled that of immature large lymphocytic cells. Finally, there were encountered cases in which the lymphosarcomatous tissues were composed predominantly of free cells, whether of the immature or of the mature lymphocytic type. These three types were termed, for descriptive purposes, "reticular," "intermediate" and "lymphocytic," respectively.

These types were found to correspond in their essential morphologic features to the immature, intermediate and mature cells resulting from normal differentiation of the cytoplasmic reticulum along lymphopoietic lines. This similarity, together with evidences of the progressive transformation of the less mature into the more mature cell types in lymphosarcomatosis, indicated that the histogenesis of this disease consists of progressive lymphopoietic differentiation of the cytoplasmic reticulum. This process is confined chiefly to the cytoplasmic reticulum of lymphatic tissue, viz., lymph nodes, gastro-intestinal tract, etc.

Lymphopoiesis as it occurs in lymphosarcomatosis manifests blastomatous characteristics. These are indicated by the aggressiveness of the tumor masses and the atypical character of the cells.

Lymphosarcomatosis arises in a region of lymph nodes, from which it extends to other regions of lymphatic tissue and other organs in progressive fashion. This spread occurs by direct local extension and by metastasis via the lymphatics and the blood stream. In addition, there occurs autochthonous formation of lymphosarcomatous foci in many centers of lymphatic tissue. This autochthonous origin is evident in partially involved nodes, where intermediate stages in the formation of these foci from local reticulum cells may be observed, and in the diffuse involvement of the malpighian follicles of the spleen in two of our cases.

As a result of these modes of spread many cases of lymphosarcomatosis show, in their late stages, a widespread involvement of the lymphatic tissues (with the exception of the spleen) and of other organs.

The origin of lymphosarcomatosis simultaneously in various lymph nodes in one region, the autochthonous mode of spread and the tendency toward restriction to one type of tissue separate this disease from true sarcoma. Lymphosarcomatosis bears certain resemblances to lymphadenosis, such as identical histogenesis, restriction to lymphatic tissue and systematization. Nevertheless, the focal origin of the former, the more aggressive character of its growth, the focal involvement of lymph nodes and the limited systematization serve to characterize it as a blastomatous disease of lymphatic tissue in contrast to the hyperplastic character of lymphadenosis.

From an oncologic point of view lymphosarcomatosis may be classified as a blastomatous disease in the group of hemoblastoses.

DISCUSSION

HENRY S DUNNING I should like to ask two questions first, what part the histiocyte plays in these tumors, if any, and second, whether Dr Ehrlich believes that the histiocyte is also formed by a reticulum cell.

J C EHRLICH In answer to the first question, what part does the histiocyte play, we have taken special note of cells which could be recognized as histiocytes in lymphosarcomatous tissue because of included phagocytosed material, and there was no instance in which such cells, whether they were in the follicles or in the pulp or in the marginal sinuses of the lymph node, appeared to play any part whatever. There were numerous cases in which the lymphosarcomatous tissue appeared punctured by large pale histiocytes which showed no evidence of participation in the formation of lymphosarcoma cells.

In answer to the second question, histiocytes are considered a part of the reticulo-endothelial system. If you want to ask, "Are these histiocytes formed by reticulum cells?" I should say, "Yes, I believe they are, but most frequently they are derived from the littoral cells or the endothelial part of the reticulo-endothelial system."

VISCERAL NEUROGLIIC SARCOMATOSIS SEATON SAILER

A rapidly fatal tumor in a woman 54 years of age, arising in the root of the mesentery and involving the visceral and parietal peritoneum and pleura, is reported.

DISCUSSION

NATHAN CHANDLER FOOT It seems to me very important in the study of these tumors to distinguish between those of Schwann cell origin and those of fibroblastic origin, and I think that one of the chief obstacles to making this distinction has been the disinclination of pathologists to get away from the old tried and true hematoxylin-eosin stain. It is important to use a trichrome stain such as Dr. Sailer used to differentiate roughly between the fibrous tumors and the nerve sheath tumors, the tumors of the sheath of Schwann. It seems quite possible that if one used a little more complicated staining technic one would be able to find out the histogenetic origin of these tumors and to distinguish between those which come from the perineural fibrous tissue and those which come from the sheath of Schwann, as this one apparently did. One has, on one hand, a slow-growing group of singly occurring tumors which, if they are not completely extirpated, are apt to recur and with each recurrence become more malignant until they are finally sarcomas. The question arises whether they are related to the multiple tumors Dr. Sailer has just described, on the other hand. It is quite possible that both camps may be right, that is, that Penfield's contention that neurogenic sarcoma is of fibrous origin and the contention of the French school that it is of Schwann cell origin may both be right under certain circumstances, and that by using more careful staining technic it will be possible to find out which tumors have their origin in Schwann's sheath and are therefore really neurogenic, and which are merely fibrosarcomas occurring along the course of nerves.

SHELDON A. JACOBSON Some time ago at the Hospital for Joint Diseases we had a tumor in which the following observations were made. The main tumor was a mass which occupied a completely destroyed lower dorsal vertebra and extended also into the vertebrae above and below. There was a marked compression of the spinal cord which had given rise to symptoms of transverse myelitis. The ileum was the site of some polypoid nodules on narrow peduncles, the largest of which was about the size of a plum. In the mediastinum was a rather large nodule which was poorly demarcated, infiltrating the wall of the aorta and completely penetrating that organ to be seen lying as naked tumor material within the lumen. On microscopic section the tumor presented an appearance very similar to that in the photomicrographs shown by Dr. Sailer. We made a final anatomic diagnosis of schwannoma in the sense used by Masson, and let it go at that.

Regular Meeting, March 28, 1935

WILLIAM C VON GLAHN, *President, in the Chair*

IRVING GRAEF, *Secretary*

A CASE OF THROMBOLYMPHANGITIS OF THE THORACIC DUCT ASSOCIATED WITH ABDOMINAL SYMPTOMS NECESSITATING EXPLORATORY LAPAROTOMY S H POLAYES

A Puerto Rican, 55 years of age, was seized with persistent abdominal pain which radiated from the umbilical region to the rest of the abdomen. Several years previously he had had epididymitis, which lasted several months, and for the past three months he had been having vague abdominal pains.

On admission to the hospital the provisional diagnosis was ileitis (influenza) and acute pancreatitis. An exploratory laparotomy failed to reveal more than marked congestion of the terminal ileum. The postoperative reaction was poor, and the patient died on the third day after admission, following a sudden rise in temperature from 99 to 105.5 F.

The blood on admission showed 13,800 leukocytes, of which 88 per cent were polymorphonuclear cells and 12 per cent were lymphocytes. Twelve hours later the leukocyte count dropped to 5,850, of which 94 per cent were polymorphonuclears. The chemical findings in the blood were normal.

Urinalysis showed numerous red cells and a trace of albumin.

Postmortem examination showed that the most important abnormalities were confined to the genital and lymphatic systems. The epididymis was the seat of chronic inflammation, which was complicated by thrombophlebitis of the pampiniform plexus. The surrounding lymphatics were apparently involved, as evidenced by marked acute lymphadenitis of the lateral and preaortic lymph nodes. The cisterna chyli was filled with purulent exudate, which extended all along the thoracic duct, presenting a marked but nonoccluding thrombolympfangitis of the duct extending to its termination in the neck, where it was lost in a mass of suppurative lymph nodes. The purulent process spread from the duct to the mediastinum and pleura, both of which were the seat of a purulent exudate. *Streptococcus haemolyticus* was recovered in pure culture from the contents of the duct as well as from all the other purulent areas and from the pampiniform plexus.

The final anatomic diagnoses were as follows: chronic epididymitis, suppurative thrombophlebitis of the pampiniform plexus, acute suppurative lymphadenitis (lumbar, thoracic, mediastinal and cervical), suppurative thrombolympfangitis of the thoracic duct, suppurative mediastinitis and pleuritis, confluent lobular pneumonia and old apical tuberculosis, a laparotomy wound with local peritonitis, cortical cysts of the kidney (arteriosclerotic), diverticulum of the duodenum.

The review of the literature reveals the rarity of the condition. Pappenheimer referred to a total of ten cases reported in the English, French and German literature up to 1921. Von Glahn in a similar review in 1924 described a case of his and called attention to a case reported by Warthin and another by DeForest, both of which, he stated, were omitted from Pappenheimer's collection. Kryloff, as well as Wurm, each added a case of his own in the period from 1927 to 1928. This makes a total of eighteen, including the case reported now.

More than three fourths of the cases reported occurred in males. In about one fourth of the cases the organism which was recovered was *Str. haemolyticus*.

DISCUSSION

WILLIAM C VON GLAHN. Several years ago I observed a somewhat similar case of suppurative lymphangitis of the thoracic duct, the cause of infection being an abrasion of the left thigh. The organism in that case was also *Str. haemolyticus*.

MULTIPLE ANEURYSMS, PROBABLY SYPHILITIC, ASSOCIATED WITH RHEUMATIC CARDITIS A ROTTINO and HENRY J SPENCER (by invitation)

This report concerns itself with a remarkable case in which was found universal involvement of the arterial tree by medial necrosis and fibrosis with the formation of numerous dilatations or aneurysms

Though the literature was reviewed for forty years back, no similar report was discovered

A 49 year old woman entered Bellevue Hospital with progressive dyspnea, weakness and loss of vision beginning insidiously six months before. A history of syphilis and rheumatism was absent. Besides signs of congestive failure, pulsating aneurysms in the neck, upper and lower extremities and aorta were found. Weakness was the prominent symptom. Death occurred in seventy days.

At necropsy the heart and arterial tree were of principal interest. In addition to cardiac hypertrophy there were thickening of a few chordae tendineae and small aneurysms at the closing margins of the aortic and mitral valves. The ascending aorta was transformed into an aneurysm with its walls thickened in places and thinned in others. Linear wrinklins, irregular scars and raised hyaline plaques affected the intima not only of the entire aorta but also of the pulmonary and all other arteries throughout the body. The aneurysms seen clinically were dilatations of thinned vessels of the neck, of the abdominal viscera and of both extremities. Aneurysms were found also affecting the ophthalmic arteries.

The microscopic lesion consisted of a medial necrosis destroying in a wholesale manner the elastic lamellae. There were extensive areas of replacement fibrosis associated with vascularization and round cell infiltration. In the intima fibrous plaques of varying sizes and thicknesses had formed. The vasa vasorum in the adventitia had thick walls and narrow lumens. The aforementioned changes were seen in all vessels described grossly as changed. Active valvulitis without verrucae was seen in the mitral valve. Aschoff bodies were found in the myocardium.

From the gross and microscopic appearance of the aortic lesions one would be entirely justified in ascribing the disease to syphilis. One might, however, raise an objection, since it is unusual to see such extensive involvement in this disease. To explain the aneurysms of the aortic and mitral valves on the same basis would be running counter to the opinion that syphilis does not attack valves primarily. The presence of the rheumatic type of lesion may well be a coincidence. If it has a meaning, this must for the present remain obscure.

DISCUSSION

ANDREA SACCONI I should like to ask whether the blood cholesterol was studied in this case, and whether sections have been stained for fat, because I encountered a case of multiple aneurysms in a girl of 18 years in which cholesterol was found in the intima of the arteries, more or less diffuse, so that in cases of this type the disturbance in the metabolism of cholesterol must be considered, and I think it is important to make a cholesterol determination.

S H POLAYES Was mention made of spirochetal studies?

A ROTTINO Though sections were stained by the Levaditi method, we were unable to demonstrate the presence of spirochetes.

A study of the cholesterol metabolism was not made clinically. The sections showed no cholesterol crystals. Preparations stained with scarlet red and sudan III revealed little or no fat in the aorta.

THE VISCERAL PATHOLOGY IN SCARLET FEVER HENRY BRODY (by invitation) and LAWRENCE W SMITH

This is a study of sixty-one autopsies in cases from the Willard Parker Hospital the Department of Hospitals, New York, forty-five of which were definitely cases of scarlet fever, the remainder probably so. In this paper is presented a

study only of the nonlocal visceral lesions, a detailed discussion of the cardiac pathology being omitted

Nonsuppurative, toxic complications of scarlet fever, particularly in the kidney and liver, have long been recognized by the clinician. The underlying pathologic picture has been particularly well described for the kidney and for other viscera. We have been especially impressed, in this study, with the frequency with which a certain type of lesion occurs in the various organs from persons dying of scarlet fever. Extensive lesions of the type to be described, the indubitable immediate cause of death, have not been frequent, but the somewhat less extensive and early lesions are common. The lesion is an interstitial one, consisting of an exudate mainly of round cells. These cannot be said to be of one type. Perhaps, by and large, lymphocytes predominate. Plasma cells are numerous. Other types of less easily classified round cells occur in considerable numbers. Giant cells are practically absent. Polymorphonuclears occur, but for the most part in small numbers, and few of these are eosinophils. The lesion has been found in almost all of the body tissues. The exact nature varies with the location. This is consequent on the fact that the interstitial nature of the lesion is not primary. It is not, we believe, the result of direct injury to interstitial tissue by the local action of metastatic streptococci or by a blood-borne bacterial toxin. We feel that it represents, primarily, a widespread injury to vascular endothelium with secondary development of fluid and cellular exudation.

All the available material at the Willard Parker Hospital from all of the autopsies on patients dying of scarlet fever or whose condition was suspected of being scarlet fever is presented. In addition, a number of cases of streptococcic infection, either primary or secondary, were also studied for comparative purposes. The material available for study was the routine autopsy sections. These had been fixed in Zenker's fluid and stained with tetra-brom-di-chlorfluorescein plus methylene blue, the routine laboratory stain. A description of the lesions found in the various organs follows.

Heart—Cardiac lesions of varying severity occur in over 90 per cent of the cases. These fall into three overlapping types: (1) either a focal or a diffuse interstitial infiltration by mononuclear cells, having no apparent distribution with reference to the cardiac blood vessels. This type is rare except as it occurs with either of the next two, (2) an infiltration in or about the smaller coronary arteries, taking the form of an arteritis or a periarteritis, in which the invading cells are mononuclear, although in some cases there occurs an admixture of polymorphonuclears, some of which occasionally are eosinophils, (3) the commonest finding, consisting of an infiltration beneath the endothelium of coronary veins, the endocardium or the endothelium of the thebesian vessels.

Kidney—The kidney is both congested and edematous and shows marked tubular degeneration. The outstanding lesion is interstitial nephritis. This consists primarily of a mononuclear interstitial exudate in the boundary zone between the cortex and the medulla. Possibly, as Schridde suggested, there is a preinfiltrative stage with accumulations of numbers of mononuclear cells in the tubular capillaries. The earliest certainly recognizable lesion is an accumulation of numbers of cells about the long veins of the boundary zone without any interstitial infiltration. More extensive lesions definitely appear to spread from these sites. Secondary foci occur, for the most part, beneath the capsule and occasionally beneath the pelvic epithelium. In the very extensive conditions the picture resembles, both grossly and microscopically, lymphatic leukemia.

Adrenal—The adrenal is almost always congested, the medulla more markedly than the cortex. Hemorrhage occurs. Cortical granular degeneration is frequent. Again, to us the striking change is an infiltration of the walls of the medullary venous sinuses by mononuclear cells, spreading to a degree through the medulla. This infiltration rarely spreads into the zona reticularis, though a case with diffuse, though not intense, cortical involvement was seen.

Liver—The liver is markedly congested and frequently shows central fatty degeneration. Edema, often extreme, is usually present. Again, mononuclear interstitial infiltration in the portal region occurs, and in many cases this appears to be definitely derived from the portal veins and their capillary branches.

Spleen—Acute splenic tumor is common. Microscopically, there are congestion and edema, with widely distended sinusoids, containing few polymorphonuclear cells. The splenic follicles are either hyperplastic or show necrotic centers. Again, infiltration of the vein and sinusoidal walls is prominent.

Other Organs—Similar lesions about veins and capillaries have been seen in the pancreas, lung, pituitary, testis, tissues of the pharynx, regional and distant lymph nodes, salivary glands and aorta.

Bacteria are not found associated with these lesions, although the blood culture is most frequently positive. It is believed that the picture represents a reaction to a circulating bacterial toxin, acting primarily on vascular endothelium.

DISCUSSION

PAUL KLEMPERER How often did you find thrombosis in the smaller or larger blood vessels?

HENRY BRODY It was very infrequent.

ALFRED PLAUT Were there any necrotic changes in the walls of the vessels, or deposits under the intima?

HENRY BRODY I do not think we ever saw necrotic changes in the walls of the vessels. We may occasionally have seen hyaline changes, but these were not particularly associated with the lesion.

ALFRED PLAUT I asked that question because I have seen in a young child whose age I do not recall a general inflammatory infiltration nearly identical with those you showed, but in the case of which I speak there were multiple necrotizing arterial lesions. It was not a case of scarlet fever.

MENDEL JACOB I noted that Dr. Brody seemed to stay away from the question of hemorrhages. He casually mentioned that hemorrhages in the adrenal occurred. Some years ago I examined some cases of scarlet fever post mortem, though I did not study them to the extent that Dr. Brody examined his, and in association with these lesions I noticed in the kidney and liver frequent hemorrhages, petechial in character. I could not find thromboses except in isolated instances. I should like to ask whether a study was made in regard to the frequency of hemorrhages, and what happened to the elastic tissue in these various vessels.

HENRY BRODY We were rather surprised to find that hemorrhage occurred as infrequently as it did. We found hemorrhage in the adrenal oftener than in any other organ, perhaps it was present in less than 10 per cent of our cases. I have only seen it once in the kidney. In the literature adrenal hemorrhage is described as a complication of scarlet fever.

We have not studied the walls of the vessels in detail.

PATHOLOGY OF B VIRUS INFECTION ALBERT B. SABIN (by invitation)

The B virus was isolated from the spinal cord and spleen of a human being with acute ascending transverse myelitis which followed the bite of an apparently normal rhesus monkey. The human lesions attributable to the virus included necrotic vesiculopustular lesions on the fingers at the bitten sites, necrotic foci in the regional lymph nodes and spleen, widespread necrosis in the adrenals and lesions in the central nervous system involving chiefly the spinal cord and medulla.

The B virus can be distinguished from all known viruses by its biologic and immunologic properties. It is pathogenic for rabbits, rhesus monkeys and, to a lesser degree, for guinea-pigs and mice. There is some evidence that it is indigenous to rhesus monkeys.

In the rhesus monkey the B virus produces lesions characterized by proliferation, the formation of acidophilic intranuclear inclusions and later cellular necrosis and inflammatory reaction. Lesions can probably develop in every tissue or organ but under the experimental conditions of this study they were observed in the skin, peritoneum and omentum, liver, spleen, adrenals, ovaries, lymph nodes and nervous system. Prominent among the lesions is specific necrosis of the blood vessels. Intravenous inoculation of the virus gives rise to an exanthem and an enanthem. Intracerebral inoculation is rapidly fatal, while after peripheral inoculation there are no signs of an involvement of the central nervous system and the animals survive. The disease in the rabbit is similar to that in the monkey except that adhesive peritonitis does not follow intraperitoneal inoculation, and peripheral inoculation leads to constant ascending infection of the nerve axis.

PATHOLOGY OF GIANT TUBERCULOUS CAVITIES LEWIS E. SILTZBACH (by invitation)

Eleven instances of complete cavernous destruction of a tuberculous lung were studied. Such lungs show either a single enormous cavity extending from apex to base or complete cavitation of each lobe with persistence of the intervening interlobar adhesion. The lung is generally markedly shrunk and is surrounded by dense fibrous pleural adhesions. The opposite lung contains relatively fresh tuberculous lesions occupying limited areas. In all eleven instances the process of complete cavitation was left-sided and occurred exclusively in females. In the literature nine clinical reports of cases of this form of pulmonary tuberculosis, with one exception, record involvement of the left lung. Two lungs showing complete cavernous destruction were examined microscopically in their entirety. In the microscopic remains of the collapse-indurated parenchyma, which were present only at the base of the cavity, innumerable lymphocytic nodular aggregates as well as a few scattered miliary tubercles were found. A surprisingly large number of small encapsulated caseous and calcified nodules of old caseous pneumonia and caseous bronchitis were also observed. Many of these old lesions showed reactivation of the tuberculous process as well as erosion and discharge into the lumen of the cavity.

The cavity is lined by nonspecific granulation tissue, and the wall of the cavity only rarely contains evidence of tuberculous change. Areas were encountered where no definitely formed wall of a cavity was present, and here the remains of lung tissue or even the fibrous pleural adhesion lay bare in the lumen of the cavity, undergoing severe nontuberculous purulent necrosis. The dense fibrous tissue of the pleural adhesion showed a rich network of elastic fibrils, probably originating in response to the forces of respiratory movements. The tracheo-bronchial lymph nodes contained numerous fresh tubercles as well as old and encapsulated caseated and calcified foci.

Regular Meeting, April 25, 1935

WILLIAM C. VON GLAHN, *President, in the Chair*

IRVING GRAEF, *Secretary*

SPINDLE CELL SARCOMA ARISING IN A FISTULOUS TRACT SOLOMON WEINTRAUB and (by invitation) JOSEPH G. LEVI

The following report is of interest because of the rarity of the condition, the unusual location of the primary tumor, and the question of etiology.

A colored man, aged 40, single, a chauffeur, was admitted to Harlem Hospital on Feb. 26, 1935, for discharging sinuses about the rectum with old perianal tracts.

In the spring of 1934 the patient noticed a small "pimple" near the anal orifice, which he opened several times with a sterilized needle. Following this he had a succession of small "boils" in the perianal region which opened and left discharging sinuses surrounding the posterior commissure of the rectum.

In November 1934 he was operated on for a horseshoe-shaped fistula. The internal opening was not found at that time, but the tracts around the rectum were dissected out. He returned for dressings, and on several occasions the surgeon was said to have removed "proud flesh" from the site of the operation.

His parents are living and well, and ten brothers and four sisters are all alive and in apparent good health.

On examination the local area presented granulating tissue and discharging sinuses on both sides of the rectum. Four days later he was operated on. The internal opening of the fistula was located and the usual operation performed. The postoperative course was uneventful, and the man left the hospital in ten days apparently cured. He was to return for dressings. It was noticed that the areas of operation were gradually filling up with what appeared to be exuberant granulation tissue. This tissue in five weeks grew from a flat elevated tissue to a cauliflower-like mass about the size of an orange. He was then rehospitalized.

During the interval between the two admissions the patient felt well, he had no loss of weight, no tenesmus, no obstructive symptoms and no bleeding but complained of the discomfort from the presence of the mass on sitting and walking.

Digital and proctoscopic examination revealed no ulceration or other changes of the rectal mucosa and no internal masses. There were no enlarged glands. The external mass was elliptic and measured 11.5 by 6.2 cm. It was covered by crusts of dry exudate and appeared to arise from the area of the dissected tract, following exactly the lines of incision in the left perianal region, crossing the posterior commissure, and then continuing for a short distance on to the right side.

Biopsies were taken from five different areas of the tumor. Roentgen examination of the bones of the pelvis, both shoulders and the chest showed no abnormalities. The Kahn and Frei tests were negative.

Microscopic examination showed a large collection of spindle-shaped cells conforming in all respects to the spindle-shaped cells seen in sarcoma. There were collections of small round cells and a few polymorphonuclear cells, showing secondary inflammatory invasion.

We could find only two references in the literature to sarcoma arising from fistulous tracts, that is, if we disregard the usual rectal and anal sarcomas. Malherbe recorded "sarcoma of the ischio-rectal fossa in a woman of 50 with also a primary sarcoma of the buttocks."

The etiology of this primary sarcoma raises the question whether the tumor arose from the granulation tissue or directly from fistulous tracts that had undergone malignant changes, or whether inflammation was an etiologic factor in the lesion. Dr. James Ewing treats this question as follows: "Many clinical observations point to the development of sarcoma from granulation tissue. It seems highly probable also that sarcoma, like carcinoma, arises through exaggerated inflammation and regeneration overgrowth of tissue cells."

DISCUSSION

LIONEL S. AUSTER: Were further biopsies made on tissue taken from different areas, and is there any roentgen evidence of the site of origin?

JOSEPH G. LEVI: Biopsies were made on specimens taken from three different sections of the tumor, but no x-ray picture to find out the origin was taken, only one to determine whether these were metastases. It appears as if it arose entirely from the fistulous tract. The tumor surrounds the anal orifice on the left side, goes around the posterior commissure and down on the right side where the tracts were excised. I wonder if in any way the fistulas were the etiologic agent.

LIONEL S. AUSTER: That brings out the question I had in mind. Every once in a while there have been reported in the literature instances of so-called sarcoma

of the skin in which on closer examination of the picture or the slides one can definitely demonstrate that the tumors are epithelial growths which have taken on a somewhat spindle-celled appearance. In some of them there has been evidence of histogenesis from the basal layer. They look like spindle-cell sarcoma, but in some areas there is a definite epithelial structure, and in view of the fact that this patient had sinus tracts, the tumor might have originated in the lining of one of these, stimulated by the infection and trauma.

SOLOMON WEINTRAUB I think the epithelial-like cells which are present here really represent a secondary infection. A number of round cells can be seen in the microscopic slide, but there is no evidence of this in the photograph. There was a superimposed infection.

METHODS OF GRADING MAMMARY CARCINOMA COMPARED WITH THE CLINICAL OUTCOME **LAWRENCE SOPHIAN** (by invitation)

All records of cases of carcinoma of the female breast at Roosevelt Hospital in which a radical operation had been done and a clinical follow-up of ten years or longer was available were analyzed histologically. There were 124 such cases. Examination revealed that 69 per cent of the patients had metastatic involvement of axillary nodes at the time of operation. Following the example of Haagensen, I graded the cases separately by growth characteristics: size of nuclei, variability in nuclei as to shape and size, adenoid arrangement, frequency of mitosis, presence of secretion, degree of fibrosis and hyalinization, and number of lymphocytes invading the tumor. When papillary formation, comedo type of growth or gelatinous degeneration was present note was made of it. Each factor mentioned was in each case given a number, 1, 2 or 3, indicating the degree of departure from normal and, presumably, the degree of malignancy. The size and shape of the nuclei were determined comparatively by making camera lucida drawings under a magnification of 400 times for each slide. The nuclei were thought to be more sharply outlined and less subject to artefact than the cytoplasm. Small nuclei were graded 1 and large ones 3. Those of uniform shape and size, whatever their size, were likewise graded 1 as to variability. Departures from this constancy were graded 2 and 3. Adenoid arrangement was accorded only two grades since well formed and constant gland formation was rarely found. The frequent, although inconstant, presence of adenoid structure was graded 1-2 and the absence of adenoid structure 3.

Secretion was estimated by vacuolation and clarity of cytoplasm, and its presence was noted by a grade of 1 if abundant and 2 if moderate in amount.

Mitoses were roughly counted by high power fields. If several were present in each field grade 3 was given. If a mitosis was found in practically every field the grade was called 2, and if there was difficulty in finding any mitoses the grade was called 1.

Fibrosis was graded 1 if abundant, with hyalinization present, and 3 if scanty and without hyalinization. Lymphocytic infiltration was graded 1 if abundant and 3 if scanty, in view of the theory of MacCarty that these are defensive factors.

These comparisons brought out the following facts. The percentage of axillary metastases was definitely higher in grade 3 than in grade 1 in regard to nuclear size, nuclear variability, secretion, adenoid arrangement, number of mitoses and lymphocytic infiltration. The greatest variation was in the grading by adenoid arrangement, in which almost twice as great an incidence of axillary metastases was found in the group of carcinoma simplex as in that of adenocarcinoma.

In the relatively homogeneous group of cases without axillary metastases there were a higher percentage of living patients and a longer span of life for the patients who died in the group graded 1 than in the group graded 3 in regard to nuclear size, nuclear variability, adenoid arrangement, secretion, mitosis and lymphocytic infiltration. The grading by fibrosis showed the most favorable cases in grade 3.

In order to combine the factors found useful in grading, the total grade was obtained by adding the individual grades together for the four major criteria—nuclear size, nuclear variability, adenoid arrangement and mitotic frequency. Since these could each range from 1 to 3 the totals could range from 4 to 12. Favorable

factors such as abundant secretion or papillary arrangement or comedo formation were given some weight by reducing the total by 1 for each such factor. The total grade was then given as 1 when the sum of all factors was less than 7, as 2 when the sum was from 7 to 9, and as 3 when it was 10 or more. When the clinical cures in the 124 cases followed ten years or longer were charted by these groups it was found that 34 per cent of the patients in the grade 1 group were alive at the end of the period as against 19 per cent of those in the grade 2 group and 13 per cent of those in the grade 3 group. The influence of the presence of more cases in which there were no axillary metastases in the grade 1 group is seen if only the cases in which there were axillary metastases at the time of operation were charted. After ten years only 7 per cent of the patients were alive in each of the three grades. The length of life was, however, greater in the group with grade 1 carcinoma than in that with grade 2, and in that with grade 2 than in that with grade 3.

The importance of grading, therefore, is greatest in determining the prognosis for cases without axillary metastases. The favorable influence of adenoid arrangement on clinical outcome is due to the relative infrequency of axillary metastases at the time of operation in this group.

DISCUSSION

ROBERT CHAMBERS. What is the relation between nuclear size in these various grades of tumors and that in the normal mammary gland? In connection with the deaths due to metastases, is there any regularity as to where metastases occur?

IRVING GRAEF. Were any of these patients treated with radiation before operation?

LAWRENCE SOPHIAN. The grade 1 size of the nucleus corresponds to the size of the normal nucleus of the epithelium of the mammary gland.

ROBERT CHAMBERS. That is the largest?

LAWRENCE SOPHIAN. No, it is the smallest. I took it merely according to what had previously been written that large cells are thought to be abnormal and more malignant, and that small cells belong to grade 1. These nuclei are between 4 and 6 microns in diameter and are about the size of the normal mammary epithelial nuclei. As far as the presence of metastases in various parts in the fatal cases goes, I did not have a high enough percentage of autopsies to make a reasonable estimate, but clinically the greatest occurrence of metastases was in the pleura, the second greatest was in the liver, and the third was in the bones.

None of these patients received preoperative radiation, some received post-operative radiation.

STUDIES OF MELANOMA IN TISSUE CULTURE. ROBERT CHAMBERS (by invitation)

During the past year my associate, C. G. Grand, and I have been making a study of mouse melanoma in tissue culture, an excellent method for use in the identification of the various types of cells found in this tumor, as the cells migrate from the margin of the explant and retain their specific morphology.

Fragments of the tumor tend to impart an alkaline reaction to the culture medium. This alkalinity has an inhibitory effect on the tumor. However, the presence of any *growing* tissue in the vicinity neutralizes the alkalinity, whereupon the cells of the tumor migrate and proliferate.

Macrophages.—These are the first cells to migrate in large numbers and are heavily laden with pigment which, under high magnification, can be seen to be in the form of irregularly sized granules and clumps of granules. The phagocytic activity of these cells continues as long as they are alive, and they readily ingest carmine granules placed in the medium even when they seem to be filled with melanin granules. They have never been seen to lose their granules except on disintegration.

Fibrocytes—In a thirty-six hour culture in which out-wandering of cells is evident the fibrocytes begin to be seen. They always appear later than the macrophages. The fibrocytes, even when first seen, contain a dense mass of melanin granules clustered about the nucleus in the main cell body. The granules are seldom, if ever, in the extended cell processes.

Melanoblasts—These dendritic cells have never been seen in cultures earlier than forty-eight, and usually appear only later than seventy-two, hours after implantation. The first sign of their appearance is the extension of delicate filamentous processes from the margin of the explant. The time taken for the cell body to appear is usually a matter of several days after the processes first come into evidence. This excessively slow migratory movement is a characteristic which is maintained even after the melanoblasts have migrated into the medium. The processes are very slender and long and are frequently branched. Melanin granules are mainly in the periphery of the cell body and in the dendrites, where they tend to be collected in irregular swellings giving the dendrites a varicose appearance. The constrictions between some of the swellings are very pronounced. The irregularity in size and the clumped state of the granules in the heavily pigmented melanoblasts are not to be confused with their condition of irregularity in phagocytes and fibrocytes, in which the apparent agglutination is throughout the entire cell.

In the hundreds of mouse and in the few human melanoma cultures which have been grown in this laboratory we have never observed cells which even remotely may be identified with epithelium. All these cultures have shown a rich supply of connective tissue elements with numerous spindle-shaped fibrocytes. The experimental evidence included in this paper confirms the generally accepted opinion that the dendritic melanoblasts elaborate, while the other cells of the tumor, particularly the macrophages, ingest, melanin.

An account of this work appeared in the May 1935 issue of the *American Journal of Cancer*, p. 36.

DISCUSSION

JACOB FURTH. Could you make successive passages of the melanoma cultures and reimplant them into mice? Do macrophages perform the same role as fibroblasts in making the explant less alkaline, thus preparing it for the growth of melanoblasts? Do the macrophages come from the parenchyma of the tumor or from the blood vessels?

ROBERT CHAMBERS. In the case of implantation, that is the usual procedure for maintaining our material. The culture can be grown for weeks and then be planted back into a mouse where it produces tumor growth.

Growing cultures of macrophages will acidify the medium, having an action similar to that of the fibrocytes. We have had cultures of the buffy coat of blood planted with a melanoma, and the cells which grew from it, the monocytes, rapidly proliferated and acidified the medium.

The question of the source of macrophages is a moot point. They may originate from the blood vessels, but they are found in quantities in the interstices of many tissues, and in all our tumors we have had more macrophages than any other kind of wandering cells. In our cultures of kidney, which has a rich supply of blood vessels, very few macrophages appear, but plenty of fibrocytes, while in tumor cultures with relatively few blood vessels we always have an abundance of macrophages.

DIFFUSE SARCOMA OF THE ENDOMETRIUM. REPORT OF A CASE. LOUISE H. MEEKER and G. L. MOENCH

Endometrial sarcoma is divided into the more frequent circumscribed and the rarer diffuse type. The case we report is an example of the diffuse type involving the entire endometrium.

The surgically removed specimen was a uterus (including the cervix and with both tubes and ovaries attached). It was 100 mm. in length and 35 by 40 mm.

in diameter at the cervical end. At the fundus it measured approximately 42 by 35 mm. It had been split open, disclosing a uterine canal lined by an irregularly thickened endometrium 20 mm in thickness in some areas. The superficial portions were gray-green, apparently largely necrotic and infiltrated by purulent exudate. In the deeper portion there was a gray opaque layer about 5 mm in thickness which poorly demarcated the involved endometrium from the underlying myometrium. Microscopically the neoplastic tissue was formed by an edematous diffuse proliferation of somewhat irregular cells containing large nuclei with abundant mitotic figures. The cells, which often formed poorly defined irregular nests, were spindle-shaped in some places and in others presented multiple branches which extended to the neighboring cells. There were no endometrial glands at any point, and epithelial cells were not recognized.

The manner of diffuse growth and the type of the neoplastic cells warrant a diagnosis of diffuse round cell and spindle cell sarcoma arising from the connective tissue of the endometrium.

DISCUSSION

LAWRENCE SOPHIAN. I am somewhat familiar with the subject of sarcoma of the endometrium because about five years ago in the laboratory of Roosevelt Hospital we had two cases within about ten months of each other both of which were of mixed cell types, that is, there were irregular groups of endometrial glands embedded in tumor in which the cells were of extraordinary size, and there was also evidence of a possible teratomatous origin, i. e., foci of cartilage and possibly some striated muscle. This is the type of tumor which Wilfred Shaw described, it is the type called botryoid sarcoma, and occurs most frequently in young women and second most frequently in women past 50, beyond the menopause, both of our patients were in the late 50's, and I think that in this respect the cases correspond to the case of Dr. Meeker, with the difference that in these sections I see a pure type of growth rather than mixed types. I think possibly the origin is similar, but in these sections the overgrowth of the more immature spindle cell has reduced the structure to an apparently single cell type. Both of the patients at the Roosevelt Hospital survived the operation but died of recurrence or of metastases within the subsequent two year period.

Book Reviews

Röntgenbefund und pathologisch-anatomischer Befund bei Lungenkrankheiten Versuch einer kritischen Vergleichung By Dr med Max Verse, o o Professor der allgemeinen Pathologie und pathologischen Anatomie, Direktor des pathologischen Instituts der Universität Marburg Price, 18 marks Volume 1, text Pp 96 Volume 2, atlas, with 144 illustrations Berlin Otto Elsner Verlagsgesellschaft, 1935

The purpose of this work was to study roentgenograms of the lungs in comparison with their gross appearance in diseases with marked structural alterations Under the most favorable conditions roentgenograms were made of the lungs, which were then cut into suitable sections after hardening, and the results of the two methods compared carefully In a number of cases roentgenograms of the lungs during life were available for comparison also The study includes fifty-four cases in all, illustrating the various forms of atelectasis, local circulatory disturbances, inflammatory processes, tuberculous disease (primary complex, infraclavicular foci, acute disseminations), Hodgkin's disease and neoplasms The first volume contains the text, in which the cases and the illustrations are described and summarized according to groups The second volume contains one hundred and forty-four full-page illustrations on special paper, each with a brief legend In most cases the roentgenogram is reproduced on one page and the photograph of the gross appearance of the cut surface on the opposite page All the reproductions are good Frequently the lung is shown roentgenographically in both the more or less collapsed and the fully distended state With the text in one volume and the illustrations in the other, careful comparative study is facilitated The book will be of interest to all who are concerned in the roentgen examination of the lungs It illustrates well the advantages that result from cooperation between the roentgenologist and the pathologico-anatomist The prediction is ventured that Verse will be followed by more ambitious attempts along the same lines

Repertorio sistematico dei miceti dell'uomo e degli animali By Arturo Nannizzi Price, 100 lire Pp 556 Siena s a poligr Meini, 1934

There has long been a need for a catalog of the species of fungi Mycology is one of the least well known fields in medicine, and when the pathologist essays to determine a species he is generally at a loss as to sources of information Nannizzi's catalog, a 557 page volume in Italian printed on enamel paper and abundantly illustrated with first class reproductions, fills this want The diagnosis of each species is recorded in a paragraph, worded in telegraphic style, which avoids unnecessary wading through Italian verbiage While this language is not at the command of most American physicians, their knowledge of Latin and of general mycologic terms ease the situation and make the information readily available with only occasional use of the Italian dictionary Unfortunately, references are not included

RADIAL INCLUSIONS OF GIANT CELLS

EDWIN F. HIRSCH, M.D.

CHICAGO

Lesions simulating tuberculous or foreign body granulation tissues and containing giant cells with rosette-shaped inclusions have been observed in the human liver, lungs, spleen and lymph nodes, in subcutaneous tissues about paraffin, in scar tissues around the ducts of the mammary gland dilated with retained secretion, in the wall of dermoid cysts, in adenomyoma nodules of the uterus, in nodules of the myocardium, in scars of the capsule of the spleen, in chronic tuberculous lesions, and in certain chronic inflammations of the skin and subcutaneous tissues. The nature of these radial inclusions in the giant cells has been an enigma, and many divergent opinions have been recorded regarding their composition and the significance of the associated lesions. The historical details are reviewed later, and the immediately succeeding paragraphs describe these granulation tissue lesions in the lungs, spleen or parabronchial lymph nodes of ten bodies and in twenty-six specimens of tissues removed surgically and submitted to routine microscopic examination. The lesions with the giant cells and their radial inclusions in the specimens of tissues removed surgically were incidental and presumably had no relation to the disorders that impelled the operations.

AUTOPSY MATERIAL

AUTOPSY 1—A white man, aged 72, with a chronic infection and concretions of the urinary tract, had taken olive oil and other simple remedies by mouth. The spleen, weighing 290 Gm., had many fibrous nodules 3 to 20 mm. in diameter. These were fibrous granulation tissues containing confluent and discrete structures resembling tubercles. There were many foreign body giant cells, epithelioid cells, lymphocytes and plasma cells. The giant cells had vacuoles from 6 to 7 microns in diameter with minute spherical granules. Many of the giant cells contained one or two radial inclusions from 5 to 20 microns in diameter (fig. 1). A radial structure 15 microns in diameter appeared to be extracellular in one section.

This research was aided by the Winfield Peck and the Watson K. Blair Memorial Funds.

From the Henry Baird Favill Laboratory of St. Luke's Hospital and the Norman Bridge Pathological Laboratory of Rush Medical College of the University of Chicago.

Fibrous tissues around some of the bronchi and blood vessels of the lungs also contained giant cells with radial inclusions, and aggregates of pulmonary alveoli were filled with vacuolated fibrin and mononuclear exudate cells (fig 2). Drop-lets of oil were demonstrated in such tissues with sudan III. The tissues in parabronchial lymph nodes were similar to those in the fibrous nodules of the spleen.

AUTOPSY 2—A white woman, aged 50, died fifteen hours following thyroidectomy. In the fibrous tissues along a few of the blood vessels and bronchioles and in the parabronchial lymph nodes were giant cells with radial inclusions associated with a few lymphocytes and mononuclear exudate cells. There were no lesions in the liver and spleen.

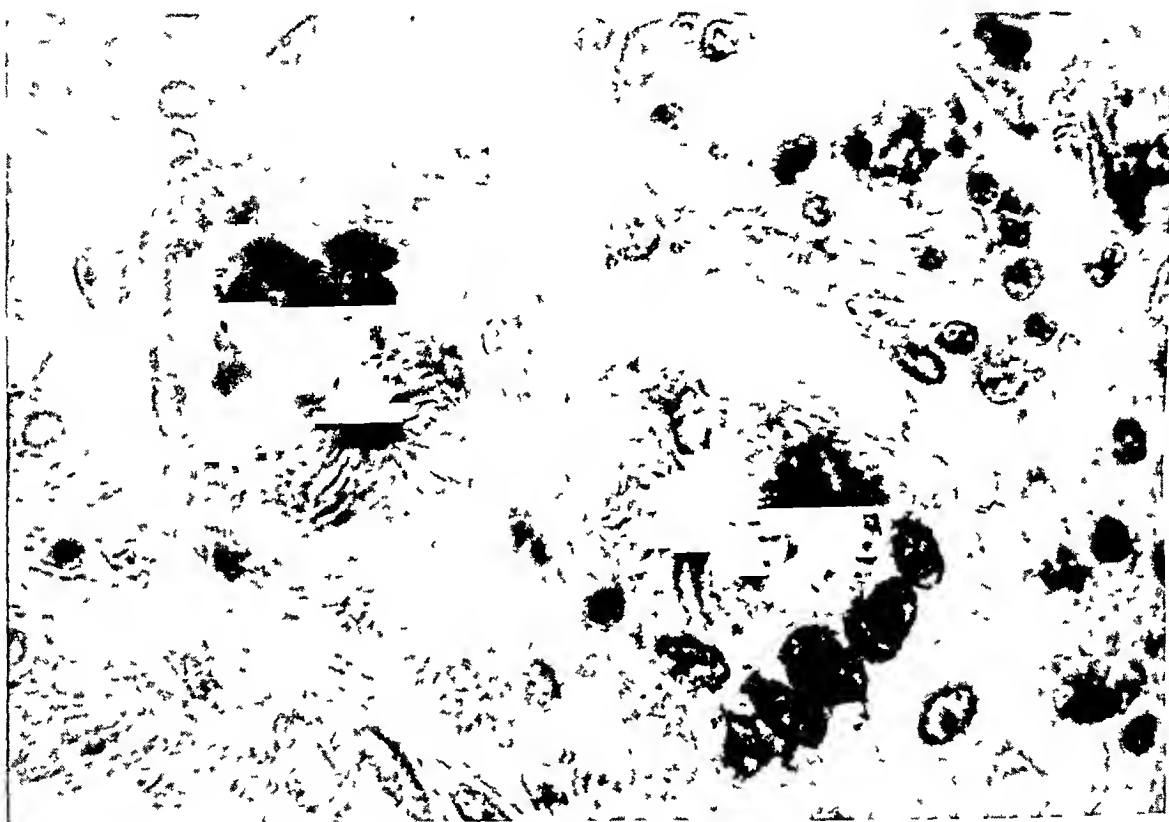


Fig 1—Photomicrograph illustrating giant cells with radial inclusions (autopsy 1). Magnification, $\times 1,260$.

AUTOPSY 3—A white man, aged 70, treated about five years for pernicious anemia, died of uremia. Small clusters of foreign body giant cells with radial inclusions were noted about the blood vessels of the lungs. The liver, spleen and lymph nodes had none.

AUTOPSY 4—A white man, aged 47, died of carcinoma of the pancreas. One of the lungs had subpleural scars containing chronic cellular exudates, and a few multinucleated foreign body giant cells in granulation tissues with cholesterol clefts. Some of the giant cells had radial inclusions. The parabronchial lymph nodes, spleen and liver had no giant cells.

AUTOPSY 5—Routine microscopic examination of the tissues in the body of a white man, aged 46, who had had hypertension and clinically and anatomically a spontaneous cerebral hemorrhage on the right, disclosed a few large vacuolated

giant cells with stellate inclusions in the parabronchial lymph nodes, but none in the sections of the lungs and other tissues examined

AUTOPSY 6—A white man, aged 72, who had had diabetes for many years, died of acute generalized peritonitis following perforated chronic ulcerative cholecystitis. He had used liquid petrolatum for constipation and an oil spray for catarrh of the nose. Routine microscopic examination of the lungs disclosed vacuolated exudates in the alveoli and bronchi. Along the margin of the vacuoles were foreign body giant cells. Similar clusters of vacuoles were discovered in the periportal tissues of the liver, in the sinusoids of the spleen and in the parabronchial lymph nodes. Sections of the spleen and liver suitably stained demonstrated that these vacuoles originally contained an oil. The tissue reaction about these droplets of oil was

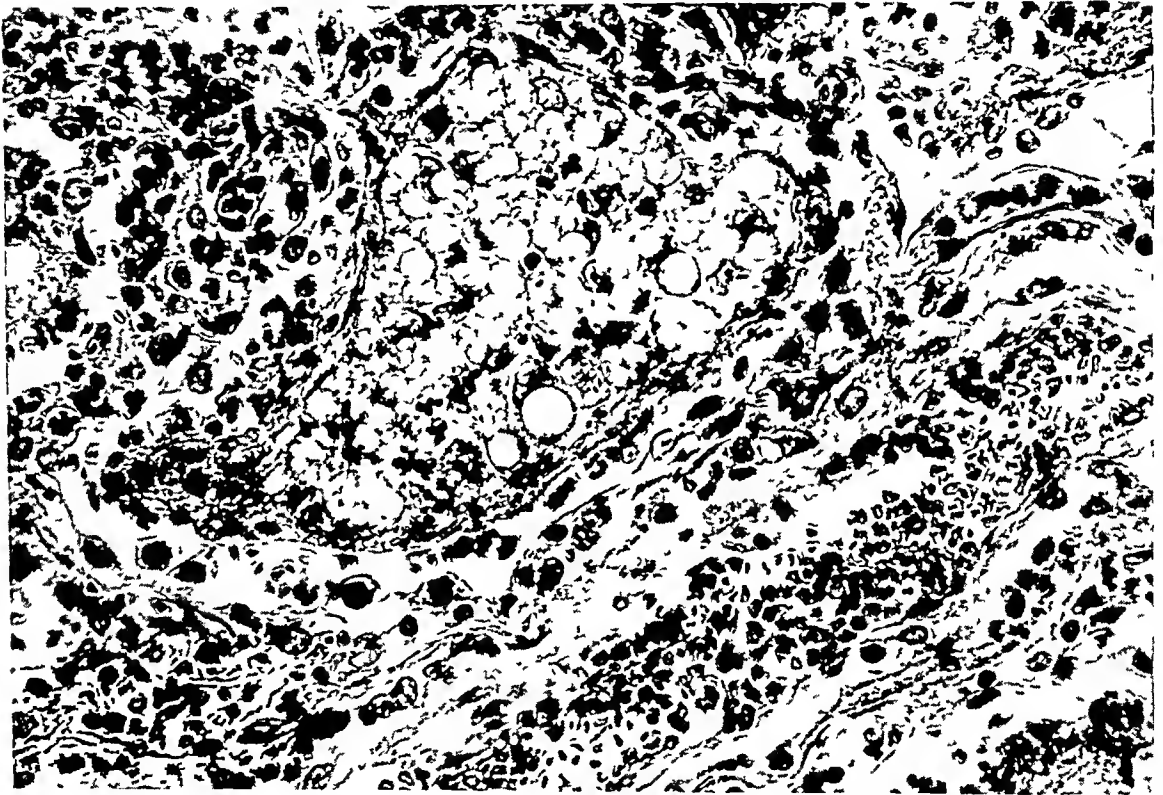


Fig 2—Photomicrograph illustrating the vacuolated exudates in the lungs in autopsy 1, presumably where droplets of liquid fat had been dissolved during the staining procedures. Magnification, $\times 620$

slight in the various places mentioned, but in the parabronchial lymph nodes were a few foreign body giant cells with large vacuoles and a large stellate inclusion.

AUTOPSY 7—An emaciated white woman, aged 39, for many years had had pulmonary tuberculosis, mainly of the left lung. Spatially distributed over three months and seven days to suit the physical conditions of the patient, three thoracoplastic operations were performed on the left posterior region and two on the anterior region. Within a few hours after the last operation the patient became unconscious, dyspneic and cyanotic and slowly died. Droplets of fat were demonstrated in the capillary and precapillary bed of the right pulmonary artery and in dilated sinusoids of the liver at the periphery of some of the lobules. The parabronchial lymph nodes had focal masses of epithelioid cells resembling tuber-

culous nodules or tissue reactions caused by fat. Apart from these lesions were a few vacuolated foreign body giant cells with stellate inclusions.

AUTOPSY 8—A Negro, aged 67, died of an infarct (thrombosis) of the brain. There was hyperplasia of the large mononuclear cells in the parabronchial and biliary (?) lymph nodes. These lymph nodes contained also a few widely scattered giant cells, some with stellate inclusions.

AUTOPSY 9—A Negro boy, aged 8 years, died of acute confluent bronchopneumonia. In the upper pole of the spleen was an encapsulated mass of necrotic tissue 4 by 0.8 by 0.8 cm. In the necrotic tissue debris were many mossy crystals with golden-brown pigment and of the size mentioned. There was no granulation tissue reaction about them.

AUTOPSY 10—The tissues of a biliary lymph node (supplied by Dr. E. R. Le Count) contained clusters of large and small vacuoles. About the vacuoles were foreign body giant cells, some with a stellate inclusion. The fibroblastic tissue reaction was slight.

SURGICAL MATERIAL

SPECIMEN 1 (*Lymph Node*)—A housewife, aged 58, had noted a painless lymph node in the left supraclavicular fossa of the neck increase within a year to 4 by 3 by 2 cm. The skin was not involved, and three other nodes, freely movable, were 1 cm in diameter. The large node was removed. The tissues were tuberculous, but also contained vacuolated giant cells, a few with one or two radial inclusions. The scar tissues had invaded the fat around the lymph node.

SPECIMEN 2 (*Thyroid Tissues*)—Thyroid tissues weighing 90 Gm with marked glandular hyperplasia were removed from a man, aged 45. In one block was a mass of scar and granulation tissues containing cholesterol slits, and a few vacuolated giant cells with radial inclusions. One radial crystal seemed to be extracellular.

SPECIMEN 3 (*Thyroid Tissues*)—Thyroid tissues with marked glandular hyperplasia and weighing 102 Gm were removed from a white woman, aged 47. In chronic granulation tissues from a necrotic portion were mononuclear phagocytes with blood pigment and several giant cells with radial inclusions.

SPECIMEN 4 (*Thyroid Tissues*)—Thyroid tissues weighing 94 Gm, a recurrent goiter, were removed from a Negress, aged 54. There was glandular hyperplasia with recent and old hemorrhages. The old hemorrhages had organized into scars containing mononuclear phagocytes with blood pigment, granular debris with cholesterol slits and a few foreign body giant cells some with radial inclusions.

SPECIMEN 5 (*Thyroid Tissues*)—A fluctuant mass of thyroid tissue weighing 60 Gm was removed from a Negress, aged 41. It was mainly an adenoma altered by hemorrhages, edema, necrosis and organization. The granulation tissues contained multinucleated foreign body giant cells, some with radial inclusions, also, coarse green and yellow threadlike masses (iron-encrusted fibers or iron-salt precipitates¹).

SPECIMEN 6 (*Thyroid Tissues*)—Thyroid tissues from a white woman, aged 58, weighed 86 Gm. Granulation tissues in an adenoma contained tissue debris with cholesterol slits and giant cells with stellate inclusions.

¹ Askanazy, M., and Bamatter, F. *Centralblatt für allg. Path. u. path. Anat.* 43: 337, 1928.

SPECIMEN 7 (*Thyroid Tissues*)—Thyroid tissues weighing 255 Gm were removed from a white woman, aged 45. There were diffuse and focal regions of glandular hyperplasia. One of the latter, 6 cm in diameter, was necrotic, hemorrhagic and partially replaced by granulation tissues. In the granulation tissues were bizarre fibrillar structures encrusted with lime or blood pigment, and among these were foreign body giant cells, some with one or more stellate inclusions.

SPECIMEN 8 (*Mammary Gland*)—A woman, aged 50, had noted a lump in the breast for five years. A retention cyst 2.5 cm in diameter, and several others, 3 or 4 mm in diameter, were in the excised tissues. A brown mass 3 mm in diameter in one of the small cysts contained granulation tissues with cholesterol slits and foreign body giant cells with radial inclusions from 15 to 20 microns in diameter.

SPECIMEN 9 (*Tonsil*)—In a crypt of a tonsil from a white man, aged 30, was a 2 mm mass of chronic granulation tissue with vacuolated giant cells containing radial inclusions.

SPECIMEN 10 (*Fractured Bone*)—A gardener, aged 40, fractured the os lunatum of his left wrist. After four months the bone was removed in several pieces. In some dense scar tissues were a few foreign body giant cells with stellate inclusions.

SPECIMEN 11 (*Uterus*)—The body of the uterus and the left fallopian tube with hydrosalpinx were removed from a multiparous white woman, aged 33. The endometrium was from 2 to 4 mm thick and had recent hemorrhages. In the interstices between the smooth muscle bundles of the stratum submucosum were small masses of lymphoid tissue containing foreign body giant cells with radial inclusions. These lesions extended from 1 to 2 mm beneath the endometrium.

SPECIMEN 12 (*Sebaceous Cyst*)—Three sebaceous cysts, from 8 to 20 mm in diameter, were removed from the scalp of a man, aged 48. Along the margin of one was a mass of foreign body granulation tissue containing cholesterol slits, some giant cells with granular inclusion material and a few vacuolated giant cells with a large radial inclusion.

SPECIMEN 13 (*Dermoid Cyst of the Ovary*)—A dermoid cyst of the ovary was removed from a Negress, aged 56. A mass of chronic granulation tissue in the wall contained mononuclear phagocytes with brown blood pigment, many large vacuolated cells and several vacuolated giant cells with stellate inclusions.

SPECIMEN 14 (*Dermoid Cyst of the Ovary*)—In the wall of a dermoid cyst of the ovary, along the edges of large vacuoles such as remain where an oil has been dissolved, were vacuolated foreign body giant cells with one or more radial inclusions.

SPECIMEN 15 (*Scar Tissues*)—Scar tissues of a urinary cystotomy fistula contained foreign body giant cells with refractile suture material and others vacuolated with one or two radial inclusions, the largest 35 microns in diameter.

SPECIMEN 16 (*Scar Tissues*)—Scar tissues from another urinary cystotomy fistula had similar giant cells. One radial inclusion was 20 microns in diameter.

SPECIMEN 17 (*Scar Tissues*)—Painful "neuroma" scars in the stump of a left forearm amputated twenty-five years before contained nerve fibers and focal exudates, including one or more foreign body giant cells, some vacuolated and with radial inclusions from 3 to 20 microns in diameter.

SPECIMEN 18 (*Scar Tissues*)—The uterus from a woman aged 43 was rough with adhesions following a preceding laparotomy. Peritoneal scars from 1 to 5 mm in diameter contained epithelioid cells, a few plasma cells and leukocytes,

some giant cells with suture material and others with stellate inclusions 15 microns in diameter

SPECIMEN 19 (*Scar Tissues*)—An old laparotomy scar adherent to the omentum was excised from a woman, aged 30. The scarred fat tissues contained foreign body giant cells with suture material inclusions and a few with radial inclusions.

SPECIMEN 20 (*Scar Tissues*)—Scar tissues removed from an old empyema of the chest of a man, aged 39, contained large vacuoles. Along the margin of the vacuoles were multinucleated giant cells, some vacuolated, others with stellate inclusions.

SPECIMEN 21 (*Scar Tissues*)—A fistula of the right epididymus in a man, aged 40, had drained for sixteen years. The excised granulation and scar tissues contained a few giant cells with refractile suture material and a few vacuolated with a single radial inclusion.

SPECIMEN 22 (*Scar Tissues*)—Nodules from the peritoneum of a Negress, aged 38, contained fat and metastatic glandular carcinoma. A mass of foreign body granulation tissue encompassed granular debris with many cholesterol clefts. There were numerous mononuclear phagocytes with brown blood pigment and clusters of foreign body giant cells. A number of these contained small stellate inclusions.

SPECIMEN 23 (*Scar Tissues*)—Periarticular fat removed from a woman about seven years after an injury to her knee contained regions of necrotic fat and granular debris. The granulation tissues around the necrotic tissues had many mononuclear phagocytes, some vacuolated and others with granular blood pigment and mossy golden-brown rosettes 10 microns in diameter. Other rosettes, from 20 to 30 microns in diameter, were encompassed by large epithelioid cells.

SPECIMEN 24 (*Scar Tissues*)—Old scar and fat tissues about fistulas of osteomyelitis of the right femur contained a few foreign body giant cells with radial inclusions.

SPECIMEN 25 (*Paraffin Scar Tissues*)—Scars of the face caused by injections of paraffin (?) seven years before excision contained large and small vacuoles. Along the margins of the vacuoles were foreign body giant cells, a few with stellate inclusions.

SPECIMEN 26 (*Paraffin Scar Tissues*)—Paraffin injected into the right groin of a man, aged 43, failed to correct an inguinal hernia. The scar tissues about these masses of paraffin contained large and small vacuoles, and along the edges of the vacuoles were foreign body giant cells, some with radial inclusions.

HISTORICAL RESUMÉ

Goldmann,² in 1890, seems to have written the first description of radial inclusions in giant cells. He saw them in histologic preparations of the wall of a dermoid cyst of the neck taken from a man, 25 years old. Ribbert examined the wall of a cyst of an omentum that Ris³ had removed and noted in the tissues clefts such as remain where cholesterol has been dissolved and giant cells with radial inclusions. The same year, De Buck and Broeckaert⁴ described inflamma-

2 Goldmann, E. E. Beitr. z. path. Anat. u. z. allg. Path. **7** 553, 1890.

3 Ris, F. Beitr. z. klin. Chir. **10** 423, 1893.

4 De Buck, D., and Broeckaert, J. Bull. Acad. roy. de med. de Belgique **17** 890, 1903.

tory reactions around paraffin in the tissues of a youth, 17 years of age. They illustrated radial inclusions within giant cells like those described by Goldmann and Ris.

Wolbach,⁵ in 1911, published abstracts on five postmortem examinations in which visceral masses of chronic granulation tissue containing giant cells with radial inclusions had been observed. The first postmortem examination was that of the body of a woman, aged 65, who died fifteen days after resection of a carcinoma of the colon. In masses of fibrous tissue along the interlobular septums of the lungs and about the bronchi and large vessels were giant cells with radial inclusions. The second examination was that of the body of a man, aged 72, with chronic urinary cystitis and carcinoma of the neck which was considered metastatic from a primary growth in the nasopharynx. Giant cells with radial inclusions were present only in the parabronchial lymph nodes. In the lungs of another man, 39 years old, miliary lesions contained giant cells with radial inclusions. Similar giant cells were in the bronchial lymph nodes. The spleen, the liver and the lymph nodes of the neck, mesentery and prevertebral tissues of a woman, aged 49, with exophthalmic goiter, had lesions containing giant cells with stellate inclusions. In the fifth instance of visceral lesions in Wolbach's report the lesions were in the lungs, spleen and liver of a woman, aged 49. Death was caused by a cerebral hemorrhage. In his discussion Wolbach referred to similar cell inclusions in tissues of a giant cell sarcoma furnished by Dr F B Mallory. Many giant cells in this preparation contained one or several hyaline spherules with radiating, delicate curved and straight spines.

Vogel,⁶ in 1911, reported lesions containing giant cells with radial inclusions in the lungs of a boy, aged 11 years. Scattered in both lungs almost completely airless, were small gray tough nodules and bands resembling miliary tubercles. Their distribution corresponded to the terminal branchings of the smallest bronchioles. No tuberculous lesions were present. Histologic examination demonstrated desquamated alveolar epithelium and obliterated terminal bronchioles. Only segments of the larger bronchioles remained, other portions were replaced by connective tissue which extended into the lumens. Giant cells with one or more radial inclusions were in the perivascular and peribronchial connective tissues. Vogel reported these as unusual foreign body giant cells with bronchiolitis obliterans. Cyanosis, dyspnea and a dry cough were some of the clinical symptoms.

In discussing the obliteration of ducts in the mammary gland and the retention thereby of fatty substances, Letulle⁷ mentioned the presence of giant cells with stellate inclusions in granulation tissues around the gland tubules. Iwanzoff⁸ observed lesions containing giant cells with radial inclusions in a large adenomyoma of the uterus of a woman aged 50. Some of the larger myoma nodules contained focal lesions the size of a submiliary tubercle, composed of lymphocytes and giant cells with radial inclusions. Ernst⁹ noted giant cells with radial inclusions in a preparation mounted in balsam, which he said resembled those described by Wolbach and others. Hummel¹⁰ observed many nodules of chronic granulation tissue with giant cells containing radial inclusions in the lungs and a few in the spleen of a woman, aged 52, who had worked for many years in a pottery.

5 Wolbach, S B. J M Research **24** 243, 1911

6 Vogel, Karl. Virchows Arch f path Anat **206** 157, 1911

7 Letulle, M. Rev de gynec et de chir abd **19** 401, 1912

8 Iwanzoff, P. Beitr z path Anat u z allg Path **52** 202, 1912

9 Ernst, Paul. Beitr z path Anat u z allg Path **53** 429, 1912

10 Hummel, Edward. Virchows Arch f path Anat **211** 173, 1913

shop A chronic cough present before she engaged in this occupation became worse Firket¹¹ saw giant cells with asteroid inclusions and cholesterol clefts in the connective tissues of an encapsulated colloid carcinoma of the neck and also in the tissues of the nose around masses of paraffin Kranzfeld¹² reported fibrous nodules containing scar tissue and many multinucleated giant cells with radial inclusions in the capsule of the spleen of a man, aged 20, who died with acute generalized peritonitis secondary to appendicitis Herxheimer and Roth,¹³ in their analysis of the finer structure and genesis of epithelioid cells and giant cells in tuberculosis, commented briefly on giant cells with radial inclusions They had observed these cells once in chronic tuberculous tissues, twice in dermoid cysts of the ovary in parts of the wall containing large cells and giant cells filled with fat and lipoids, which suggested a pseudoxanthoma, and once in lupus pernio of the skin

Diss¹⁴ recorded two lesions containing giant cells with radial inclusions One was a subcutaneous node that developed slowly and without ulceration of the skin in the forehead of a woman, aged 65 The excised tissues consisted of inflammatory nodules which had elevated and thinned the skin and had infiltrated the entire thickness of the frontal muscle In the second instance there were nodules in the myocardium of a woman, aged 46, who had died of pulmonary tuberculosis Diss, in his report, referred to an observation by P Masson of similar giant cells with radial inclusions An unsuccessful search for a record of this observation in the usual indexes of the medical literature prompted a letter of inquiry to Dr Masson In his reply he stated "When my assistant, Dr Diss, was preparing his report, I showed him various tissues containing giant cells with radial inclusions in my own personal collection, and in particular a cancer of the breast with an abundance of these cells in an old hemorrhage in the process of resorption This is the cancer to which Diss has alluded, but I have never published an account of the observation I have seen these asteroid bodies several times and always within a necrotic hemorrhagic focus, and thus in the presence of disintegrating blood which to me seems indispensable for their production"

Frothingham,¹⁵ in 1929, in a report of progressive thrombosis of the smaller branches of the pulmonary arteries in a woman, aged 38, described tubercle-like lesions of mononuclear and giant cells in the pleura and interlobular septums adjacent to blood vessels and bronchi Some giant cells contained the stellate inclusions described by Wolbach The histology and distribution of the lesions in the lungs were identical with those in Wolbach's report Similar lesions were in the liver Two illustrations of giant cells with radial inclusions appear in Mallory's text¹⁶ He stated that "spiculated bodies" had been seen in the spleen in a few instances, enclosed in endothelial leukocytes and giant cells The lesions resembled miliary tuberculosis but were without necrosis The bodies seemed chemically fibrinoid They were not the cause of the lesion, which was probably of infectious origin, but were a secondary formation Stoddard and Cutler¹⁷ observed in

11 Firket, C Virchows Arch f path Anat **215** 454, 1914

12 Kranzfeld, M Frankfurt Ztschr f Path **15** 297, 1914

13 Herxheimer, G, and Roth, W Beitr z path Anat u z allg Path **61** 1, 1916

14 Diss, A Bull et mem Soc anat de Paris **94** 349, 1924

15 Frothingham, Channing Am J Path **5** 11, 1929

16 Mallory, Frank B Principles of Pathological Histology, Philadelphia, W B Saunders Company, 1914, pp 207 and 613

Rockefeller Institute for Medical Research, 1916, p 55

17 Stoddard, James, and Cutler, E C Torula Infection in Man, Monogr 6

Torula-infected lung tissues giant cells with stellate masses, which they considered the same as those described by Wolbach in the spleen

The accounts mentioned state that the lesions containing giant cells with radial inclusions are essentially chronic granulation tissues like those caused by a foreign body. The dimensions of the giant cells vary, but are about the same as those of the Langhans' giant cells in tuberculous lesions. The cytoplasm of the giant cells is vacuolated and radial inclusions are not present in all. The inclusions seen by Ris were from 15 to 25 microns in diameter, those seen by Wolbach, from 5 to 25 microns in diameter. Fine or coarse spines radiate from a central, compact, round, oval or elongated nucleus, these are from fifteen to thirty in number, according to Vogel, or less (Wolbach). When the number is small and the inclusion large, the spines are coarse, when there are many, they are fine. A single giant cell may contain one or several. The inclusions generally are found in a clear part of the cytoplasm. The spines of some radial inclusions extend straight from the center, others are curved distally with ends bent, as though the inclusions were confined in a place less than their diameter. Wolbach alone reported extracellular radial structures.

The nature or origin of the radial inclusions is not definitely stated in various reports. Most authors limit themselves to descriptions of the appearance of the structures, the staining qualities and solubility reactions. The conclusion that all the reports concern radial structures of the same type is presumptive, but the illustrations and descriptions favor the opinion that, although they are present in various parts of the body, they are essentially the same. The original description by Goldmann referred to the inclusions as fat crystals, an opinion rather than a conclusion derived from tests. Ribbert, in the report by Ris, is quoted as having stated that the inclusions were not crystals or echinococcus hooklets but resembled molds (undeveloped spores and filaments). De Buck and Broeckaert considered them hypertrophied centrosomes or asters.

Wolbach tested the solubility of the radial inclusions in acids and alkalis and found them insoluble. He noted that the radial inclusions in formaldehyde-fixed material were not blackened with silver nitrate, were not colored with iron-hematoxylin and Weigert's elastic tissue stain and did not react with fat stains. No qualitative test for iron was obtained. The best stain was Mallory's phosphotungstic acid-hematoxylin method, which colored them dark purple. Because of this staining quality, Wolbach discussed their formation from fibrin or fibrin derivatives, but did not state definitely that this was their origin. Although Wolbach observed some of the radial structures in the sinuses of lymph nodes, he concluded because of the rarity of this and because

the small forms were in mononuclear cells and the larger only in giant cells that there was an actual increase in the size of the inclusions after they were taken up by endothelial cells, and that giant cells formed as the inclusions increased in size. Vogel used many dyes and tests in trying to determine the nature of the inclusions. He observed that only elastin stains were effective. They failed to react with stains for fat, amyloid, iron and glycogen. They were insoluble in acids and alkalis and were not doubly refractive. The Bielschowsky stain was mentioned, but the results with this technic were not recorded.

Vogel, in speculating on the cause of the bronchiolitis in his patient, wondered whether some mitant had been aspirated, causing the scarring and pneumonia, and then had crystallized in the lungs, stimulating the formation of giant cells. The medication given at home was linden tea and cod liver oil. This search for clues being unsatisfactory, Vogel considered that possibly the inclusions were derivatives of elastin, but concluded finally that they consisted of a substance with staining reactions like elastin. He also was undecided whether the radial inclusions as foreign bodies stimulated the giant cells or were crystalline products within the cells. The report by Ernst is limited to the examination of a tissue preparation mounted in balsam. He thought that the radial inclusions might be cholesterol. Letulle considered the inclusions parts of engulfed elastic tissue fibers. Iwanzoff, following a discussion of various possibilities, believed some degenerative product of cells the most likely origin of the inclusions. Then, after a comment on the similarity between Wolbach's illustrations and those by Wakabayashi¹⁸ of the astrospheres in tuberculous and other giant cells, he expressed the conviction that the inclusions were degenerated astrospheres. His material did not permit him to confirm this idea. Aschoff,¹⁹ commenting briefly on Iwanzoff's report, disagreed with Ribbert's opinion that the inclusions were undeveloped portions of a mold, but offered no opinion of his own. Hummel, from the results of staining reactions, concluded that the radial inclusions were crystalline precipitates that resembled changed elastin. Certain differences in staining reactions led him to believe that they were not elastin fibers. The "foamy" structure of the giant cells further suggested to Hummel that the origin of the radial inclusions was probably the crystallization of some unidentified substance in the cell. Firket concluded that the radial inclusions were not a retrogressive product of elastic fibers but had arisen in the giant cells through a differentiation of the mitomes, which differentiation was related to the vacuolation of the protoplasm. Kranzfeld believed that these radial structures were probably the result of a disturbance in karyokinesis or

18 Wakabayashi, T. Virchows Arch f path Anat **204** 421, 1911

19 Aschoff, L. Beitr z path Anat u z allg Path **52** 444, 1912

represented pathologic astrosphere formations Herxheimer and Roth stated that in a degenerative process of the giant cells the protoplasm becomes fluid and vacuolated. Substances probably protein in nature with the staining qualities of elastin crystallize as radial structures about a nucleus which is some other substance—possibly lipid material, liberated by the changes in the protoplasm. The idea of a lipid substance acting as a nucleus was derived from the observation that some of the radial structures had a central compact granule which could be stained by the Fischler and the Lorraine-Smith methods. Diss expressed the belief that the asteroid bodies resulted from the precipitation of a protoplasmic substance consequent to changes in the colloidal equilibrium of cellular protoplasm.

An analysis of the places in the body with lesions containing giant cells with radial inclusions discloses that they are (1) focal in tissues abundant with fat (Goldmann, Ris, De Buck and Broeckaert, Ernst [?], Letulle, Firket, Diss, Kranzfeld [?], Herxheimer and Roth), and (2) systemic in visceral tissues such as the lungs, spleen, liver and lymph nodes (Wolbach, Vogel, Hummel, Frothingham). The anomalous places where they have been observed are an adenomyoma of the uterus (Iwanzoff) and the myocardium (Diss). The distribution of the lesions in the lungs is in the peribronchial or bronchial tissues (Wolbach, Vogel, Frothingham), in the spleen, within the pulp tissues (Wolbach), and in the liver, along the portal canals (Wolbach, Frothingham).

In summary, the inclusions are reported to be insoluble in potassium hydroxide and mineral acids (Wolbach, Iwanzoff, Vogel, Herxheimer and Roth) and in all the reagents used in fixing, embedding and staining the tissues. They do not react with scarlet red (Wolbach, Herxheimer and Roth), osmic acid (Wolbach), Nile blue and Sudan III (Hummel, Herxheimer and Roth), the Fischler and Lorraine-Smith methods, excepting the central body (Herxheimer and Roth), and fat stains not specifically mentioned (Vogel). They do not contain iron (Wolbach, Vogel), amyloid and glycogen (Vogel) or mucin (Hummel). They are isotropic in polarized light (Vogel, Herxheimer and Roth), they do not reduce (formaldehyde-fixed tissue) silver nitrate (Wolbach), and they stain with elastin stains, such as fuchsin and safranin (Vogel, Herxheimer and Roth), and especially with the purple component of Mallory's phosphotungstic acid-hematoxylin stain (Wolbach).

Wolbach noted that some parts of the inclusions stained differently from others. With phosphotungstic acid-hematoxylin, a central body and the spines stained dark blue, and the material which surrounded the central body and from which the spines radiated stained pale brown. The individual spines consisted of a peripheral purple material and a core continuous with the material surrounding the central body. The tips of

the spines stained solidly. Strong reagents decolorized the central body from the periphery inward, the spines were less resistant to decolorization. The spines and central body stained deeply by the Gram method, the intervening material, with the counterstain. Herxheimer and Roth, noting that the central granule of some radial structures stained with the Fischler and the Lorraine-Smith methods, concluded that this portion was lipid.

The results of such contrasting stains demonstrating affinities for both dyes or at least a pronounced affinity of certain parts for one dye over the other, and, to some extent, the decolorization tests, suggest that the inclusion material is a complex of something with crystalline form and a substance or substances which impart staining affinities to the periphery and central mass, and that decolorizing agents such as acid alcohol and potassium permanganate followed by oxalic acid may remove the substance or substances responsible for the staining qualities but leave the inclusion largely intact.

EXPERIMENTS

Many of the microchemical tests and staining properties of the radial inclusions summarized in these paragraphs were verified with sections of the spleen tissues in the first postmortem examination described. The inclusions stained dark purple with phosphotungstic acid-hematoxylin. They did not stain with sudan III (formaldehyde-fixed tissues), they were not blackened by the Bielschowsky stain, and they did not react with the microchemical tests for iron, calcium or uric acid. The inclusions and the cell structures of the tissues lost their affinity for the purple component of the phosphotungstic acid-hematoxylin stain by leaching with normal sodium hydroxide-solution. Their shape remained intact. This suggests that the inclusions are impregnated with a substance having a marked affinity for the purple component of the stain which is not necessarily the inclusion material itself. Wolbach noted that a small spherule in the center, the edges and tips of the spines stained purple, the body portions, pale brown.

The configuration of the inclusions in the giant cells is crystalline. Goldmann, who wrote the original description, considered them crystalline fat, Vogel and Hummel believed them to be an undetermined crystalline substance. If the radial structures are crystals, and such an inference is reasonable, the crystalloid came into the tissues in solution and separated according to laws²⁰ governing crystallization. These state

²⁰ Walker, James. *Introduction to Physical Chemistry*. New York, The Macmillan Company, 1922. Shade, H. *Munchen med Wchnschr* **58** 723, 1911. Wells, H. G. *Chemical Pathology*, ed 4, Philadelphia, W. B. Saunders Company, 1920, p. 452.

that the solubility of substances in solution varies with the temperature, and that at a given temperature the solvent may be unsaturated, saturated or supersaturated. An unsaturated solvent becomes saturated or supersaturated at a definite temperature by solution of more of the substance or by concentration of the solvent volume. From supersaturated solutions the solid separates around micellae, and if a crystalloid, it is in crystals. The size and number of these depend on the speed of separation, some are single, and others are joined in a compact crystalline mass.

Solids colloiddally distributed in aqueous systems separate in laminae on the inner surfaces of containers or in concentric layers about floating micellae. Each layer of the solid colloid corresponds usually to a phase of separation. Laminated concretions form in aqueous systems even when a crystalloid as well as a colloid separates from solution, and the colloid framework is impregnated so intimately with the crystalloid that microscopically a distinction of structure is impossible. Fibrin dissolved in the minimal quantities of from 0.07 to 0.1 per cent confers the laminated structure to a concretion.

The conclusion that the crystalline inclusions of the giant cells separated from some supersaturated fluid in the tissues was derived from these laws governing crystallization. The fluid reached its state of supersaturation in the body by concentration of the solvent. Since laminated concretions form in the presence of dissolved protein, the original solvent of these radial structures did not contain appreciable amounts of colloid, and consequently the system in which the crystals formed was not aqueous. The deposits of urates in cartilage with gout may seem to contradict this statement, but the colloid content of these tissues may be small, and urates notably separate amorphous and gradually change into the less soluble crystalline form.

These theoretical conclusions were not correlated as quickly as they are here stated. Analyses of the spleen in the first postmortem examination disclosed no excess of inorganic constituents and purine compounds and the concretions of the urinary bladder and kidney were mixtures of calcium oxalate, carbonate and phosphate. Finally, in digesting 19 Gm. of moist spleen tissue for analysis a considerable quantity of lipoid material was observed on the surface of the nitric acid digestion mixture. A quantity of lipin, solid at room temperature, was extracted with chloroform. Chloroform extracts of other hydrolyzed spleen tissues contained much more of this tan-yellow, greasy lipin material. It had an odor resembling hydrous wool fat, did not react with the acetic anhydride-sulphuric acid test for cholesterol and, microscopically contained rosette-shaped crystals and needles resembling stearin and palmitin, as well as the radial inclusions of the giant cells. Some unsaturated fatty acid was demonstrated by the bromine test. The

melting point of these fractions ranged between 65 and 75 C. A lead salt insoluble in ether melted at about 147 C, but this result was not confirmed because of the small amount of material available for analysis. These lipins were recovered from many parts of the spleen, not alone from tissues with fibrous nodules. The results of these examinations and the determinations of the melting point suggested that the inclusions were probably stearin, palmitin or derivative compounds, and that these substances were responsible for the lesions in the tissues.

When neutral fats are lodged extracellularly in the body following trauma or necrosis of fatty tissues or injection, their orderly disposal is disturbed. Berner²¹ observed that subcutaneous fat about abscesses had retrogressive changes similar to those occurring with fat necrosis. First acidophilic granules appeared in the fat cells, then rosettes of crystals and, finally, masses of calcium soaps and debris. Heyde²² and Verebely²³ noted crystalline structures in the reparative changes of injured fat tissue, and chronic granulation tissues containing vacuolated phagocytes and foreign body giant cells. Abrikossoff²⁴ mentioned among the ultimate results of the liberation of fat in tissues the formation of granulation tissue with tubercle-like structures and, eventually, a scar. Embolic fat in the circulation may cause similar tissue reactions. Wuttig²⁵ injected rabbit fat (melting point, 41 C) into the liver tributaries of the portal vein. After from six to ten days he noted very little tissue reaction in the capillaries but a marked proliferation of the endothelium in the larger branches, and giant cells. The large bundles of star-shaped and feathery needles, soluble in alcohol, were considered stearin and palmitin. Smith and White²⁶ observed in fatty cells, especially of the liver, crystalline forms commonly designated as margarine, margaric acid, stearin or stearic acid, which were neutral fats and not fatty acids.

According to Corper and Freed,²⁷ mild proliferative changes occur in the lungs of rabbits following intratracheal injections of olive oil and liquid petrolatum. Laughlen²⁸ noted similar changes in the lungs of children not only when oil was introduced into the trachea but also when it was sprayed in sufficient quantities into the nose and throat. Pinkerton²⁹ reported more extensively the effect of oils and fats in the lungs of children. He³⁰ later studied experimentally in dogs and rabbits

21 Berner, O. *Virchows Arch f path Anat* **193** 510, 1908

22 Heyde, M. *Deutsche Ztschr f Chir* **109** 500, 1911

23 Verebely, T. *Beitr z klin Chir* **54** 320, 1907

24 Abrikossoff, A. *Centralbl f allg Path u path Anat* **45** 396, 1929

25 Wuttig, Hans. *Beitr z path Anat u z allg Path* **37** 378, 1905

26 Smith, J. L., and White, C. P. *J Path & Bact* **12** 126, 1907

27 Corper, H. J., and Freed, Harold. *J A M A* **79** 1739, 1922

28 Laughlen, G. F. *Am J Path* **1** 407, 1925

29 Pinkerton, Henry. *Am J Dis Child* **33** 259, 1927

30 Pinkerton, Henry. *Arch Path* **5** 380, 1928

certain phases of this problem. He found that oily substances were transported from the lungs into the parabronchial lymph nodes and into the spleen. In the various tissues they stimulated proliferative reactions. Pinkerton concluded that the original fatty acid content of a fat and the speed with which free fatty acids are formed by hydrolysis determine the reactive response of the tissues. The simple vegetable oils are bland, he stated, because they have no free fatty acids and the tissues have no specific lipases. The animal fats are irritants because moisture, warmth and enzymes in the tissues hydrolyze the fat and liberate the fatty acids. Many authors have mentioned the similarity between the lesions of tuberculosis and those caused by fats in the tissues. Sabin, Doan and Forkner³¹ produced lesions in rabbits with the acetone-insoluble lipins of human tubercle bacilli, which closely simulated those produced by actual infection with *Bacillus tuberculosis*. Saturated fatty acids were important constituents of the lipin fractions. Various authors have emphasized the similarity between the lesions of tuberculosis and those containing giant cells with radial inclusions.

Attempts to produce lesions in rabbits comparable to those in human tissues by intravenous injections of olive oil, extracted human and mutton fats, olive oil containing additional quantities of palmitin or stearin, oleic acid with palmitin or stearin, calcium or magnesium palmitates and stearates dissolved in oleic acid or in olive oil with sufficient quantities of palmitic or stearic acid to effect solution were unsuccessful. These results indicated that some chemical changes or additional substances were necessary in order that such lipin material should stimulate the growth of chronic granulation tissues. The giant cells with the inclusions described in the published reports and in the tissues of this account were observed frequently in granulation tissues having clefts such as remain where cholesterol has been dissolved. Olein, the important fat of human tissues, liquid at body temperature, holds in solution palmitin, stearin, cholesterol and other lipin substances. When cholesterol is deposited in tissues, it stimulates foreign body granulation tissues,³² and tissue lipoids containing cholesterol³³ produce lesions resembling tubercles. The presence of cholesterol or a similar substance in the lipid material seemed necessary, therefore, in order to produce characteristic granulation tissue lesions. A lipin mixture of fat extracted from human omentum with heat (90 C), fortified with cholesterol from human gallstones and palmitin or stearin,³⁴ when injected intravenously into

31 Sabin, Florence R., Doan, C. A., and Forkner, C. E. *Am. Rev. Tuberc.* **21**: 290, 1930.

32 Le Count, E. R. *J. M. Research* **7**: 166, 1902.

33 Simonds, J. P. *Am. J. Path.* **3**: 13, 1927.

34 Some of these mixtures contained a small quantity of suspended finely powdered calcium carbonate.

rabbits stimulated in the lungs foci of chronic granulation tissue with giant cells resembling closely the lesions found in human tissues. These mixtures were supersaturated with palmitin or stearin when injected, so that at the body temperature of the rabbit a certain amount crystallized in rosettes. Animals given injections were killed from two to three weeks after the first one. The lesions in the lungs had a fibroblastic stroma, epithelioid cells, lymphocytes and one or more foreign body giant cells (fig 3). Large lesions had many giant cells and clefts such as remain where cholesterol has been dissolved. Certain giant cells

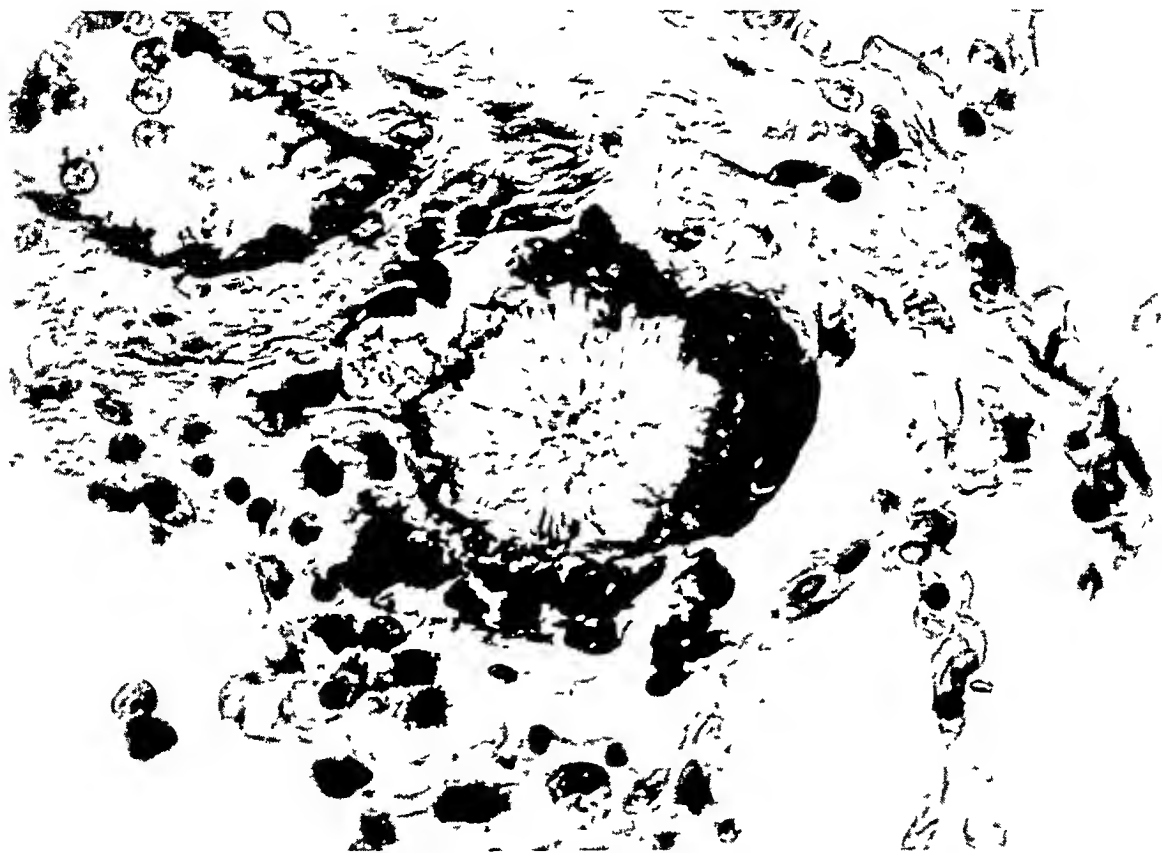


Fig 3—Giant cell produced in the lungs of a rabbit with human fat containing cholesterol and stearin. The crystallization factors in this artificial system are comparable only to those occurring in human tissues with disease. Note that much of the lipin crystal has become insoluble in fat solvents. The margins and tips of the spines have the staining qualities of elastin material. Phosphotungstic acid-hematoxylin stain, magnification, $\times 1,065$.

contained small crystals and vacuoles. Some were only vacuolated, others had engulfed or encompassed large rosette-shaped crystals, and many encircled droplets of lipin material in various stages of absorption. Where absorption of the lipid substance had progressed considerably, a clear part of the cell contained a granular material and a fairly complete radial structure. Cells with partially absorbed globules had a large

vacuole and peripheral portions of the rosette projecting radially into the cytoplasm around the vacuole. The crystals, at least those retained in the tissues sufficiently long, had become insoluble by their contact with the tissues. In sections stained with phosphotungstic acid-hematoxylin, the delicate spines were purple throughout, the coarser had a red-brown core and a thin purple margin.

These experiments, of course, indicate simply how a lipin mixture containing cholesterol may stimulate tubercle-like lesions with giant cells in tissues and that crystals of palmitin or stearin separating from such mixtures may become insoluble rosette inclusions in giant cells. Perhaps other lipin mixtures containing substances having the properties of cholesterol can stimulate similar granulation tissue reactions. The chemical tests of the lipin material recovered from the human spleen failed to demonstrate cholesterol, but these fractions were obtained from spleen tissues mainly between, and not in, the fibrous nodules. Presumably, additions of cholesterol-like substances from necrotic tissues to the lipins or chemical alterations of the lipin material occurred focally and initiated the granulation tissue reaction, or such mixtures were deposited focally and stimulated the granulation tissues wherever they lodged. The splenic pulp of rabbits that received injections of human fat containing cholesterol and palmitin had extensive regions of inflammatory tissues but no giant cells—changes unlike those of the lungs, where much of the dissolved cholesterol and saturated fat separated from solution.

The evidence favoring the conclusion that the radial structures of giant cells in systemic and focal masses of chronic granulation tissues are originally crystalline fats solid at body temperature, such as palmitin and stearin, separating from oily mixtures containing cholesterol or cholesterol-like substances, is derived from (1) the circumstance that the lesions with these giant cells, according to published reports and in the investigated tissues, have been associated with fat tissues or have contained clefts such as generally are recognized as remaining in tissues where cholesterol has been dissolved, (2) the chemical analysis of spleen tissues with such lesions whereby large amounts of lipin of the character of stearin, palmitin or mixtures were demonstrated and which on solidification formed rosette-shaped crystals, and (3) the experiments demonstrating that lipin mixtures of cholesterol with a high content of palmitin stimulate in the lungs of rabbits tubercle-like lesions with giant cells having radial inclusions and comparable to those seen in human tissues. The crystals so lodged in the tissues gradually became insoluble in fat solvents and had, in sections stained with phosphotungstic acid-hematoxylin, a purple periphery and a red-brown center. These staining qualities favor the conclusion that substances from the tissues confer

the elastin-like staining qualities to the crystalline fat, but that other changes of the crystalloid substances render them insoluble in the usual fat solvents

CONCLUSIONS

The radial inclusions of giant cells observed in tubercle-like granulation tissues are crystalline forms of fats solid at body temperature, such as palmitin or stearin, separated from an oil system containing cholesterol or substances with the physical properties of cholesterol

The formation of these crystals in a liquid fat system is according to the usual laws governing crystallization, and the factors accomplishing supersaturation of the system are mainly the abstraction of the liquid portion faster than the combustion of the dissolved solid fat

Certain chemical changes take place in the composition of the crystals in the tissues so that they become insoluble in fat solvents. Further changes or additions in the tissues produce the elastin-staining qualities

An embolic dissemination through the blood and lymph channels distributes the lipoid material into the liver, spleen, lungs and lymph nodes—visceral tissues commonly the site of systemic lesions. Lipoid, insufflated or aspirated into the respiratory passage, may initiate lesions along the bronchi and bronchioles

BASE-PROTEIN-ACID COMPOUNDS

MARTIN H FISCHER, M D

AND

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CINCINNATI

The physicochemical constitution of naturally occurring proteins, such as the protoplasm of animals or plants, eggwhite, blood plasma or milk plasma, continues to be a matter of debate, even though the purely chemical attack on the problem was initiated in the sixties of the last century and the physicochemical attack but a few years later. The opinion of the majority today still holds that these natively occurring materials are mere mixtures of two or more proteins suspended in water. Since their properties when chemically pure are in no wise those of these same proteins when encountered naturally, the effects of the inorganic acids, bases and salts constantly present in living matter or its juices have been called on to explain the difference. The accepted point of view here again is that these "electrolytes" are materials that are merely "dissolved" (in dilute solution) in the water of protoplasm and thus "influence" the behavior of the mixed proteins.

Against this point of view which, briefly stated, holds that living matter (such as any of the meats) is an aggregation of droplets composed essentially of a solution of salts in which protein is suspended, stands the simple fact that the tissues of plants and animals will not "mix" with more of their solvent (water). Even egg white or blood plasma will not do so without chemical decomposition (increase in alkalinity, precipitation of globulin). This by itself proves that the high percentage of water found in living matter is not in the same form as ordinary water, in other words, it cannot be "free" but must be held in a chemically combined form¹. Protoplasm is therefore a "hydrate". But the salts found in protoplasm (commonly obtained by ashing it) cannot easily be leached out, which proves that they too are not merely "dissolved" but held in combination. The necessary conclusion is therefore that the essence of living matter is a protein (or several proteins in combination or mixture) to which the inorganic radicals of the salts are tied chemically in the form base-protein-acid and not in the form protein-salt.

Comparative colloid-chemical studies of tissues, on the one hand, and of various proteins, on the other, confirm this point of view. Thus, pure

From the Laboratory of Physiology, University of Cincinnati

¹ Fischer, Martin H. *Edema*, New York, John Wiley & Sons, 1910, p. 184

proteins (like the globulins) take up very little water, but they swell enormously as soon as acid or alkali is combined with them. The tissues of the body, however, are more than either basic proteinates or acid proteinates. *They are both in one.* This is proved by the physiologic, pharmacologic or toxicologic action of the various salts on them. All neutral salts dehydrate the living mass but, at the same concentration very unequally. Thus the chlorides of potassium, magnesium, calcium and mercury dehydrate increasingly in the order named (and so reduce the body weight of an edematous condition and act as hydrogogic cathartics, diuretics or sudorifics in the order named), while the acid radicals, united to any base, dehydrate increasingly in the following order: chloride, acetate, sulphate and phosphate (exhibiting a like order of action when employed therapeutically).² The physiologic action of any salt comes therefore, to be compounded of the action of its constituent radicals (which makes magnesium sulphate a more powerful cathartic or diuretic than sodium chloride and makes a mercury salt the most powerful of all). The fact that *both* radicals are physiologically active is proof that *both* acid and basic radicals appear in the native protoplasm.

In colloid chemistry pure basic proteinates which are hydrated in the order of the base series here outlined, and pure acid proteinates, which are hydrated according to the acid series, have been prepared. But base-protein-acid compounds that are analogous to those existent in the living mass have not yet been produced. These paragraphs describe how they may be.

THE PRODUCTION OF BASE-PROTEIN-ACID COMPOUNDS

The production of a basic proteinate or an acid proteinate is simple. Mere addition of any hydroxide or acid to a protein will yield some of the compound, but to have the reaction complete *it must be carried out in the absence of free water.* The problem is analogous to the production of a soap from a fatty acid and an alkali. Here, too, only when carried out in concentrated form (in other words, without the presence of free water) is this reaction complete. *Utilization of the same principle on a protein allows not only a base or an acid but both to be tied to the protein.*

Under ordinary circumstances sodium caseinate or casein chloride on the addition of an acid or an alkali reverts to its pure and anhydrous form (it is said that the acid or alkali reacts with the base or acid in the caseinate, frees the casein and allows it to fall out as a precipitate).

² Fischer, Martin H., and Sykes, Anne. *Science* **37** 845 1913, *Kolloid-Ztschr* **13** 112, 1903, *Edema and Nephritis*, ed 2, New York, John Wiley & Sons, 1915, p 295.

If the same additions are made, but in a reaction mixture in which all the water is held in hydrate form (in practical terms, and for casein this means in any amount below 80 per cent of the total mixture), the acid or alkali does *not* crack off the contained base or acid, but *both* combine with the protein nucleus

The eighty per cent of water in these systems (not free but held in hydrate form) is a first value of physiologic significance to be remembered in these studies. It needs to be compared with the normal percentage of water discoverable in the composition of any of the ordinary tissues. Human blood for example, carries only about 80 per cent of water, heart muscle 79 per cent, skeletal muscle 75 per cent, the brain 74 per cent, the skin 72 per cent, the liver 68 per cent and the bones 62 per cent.³

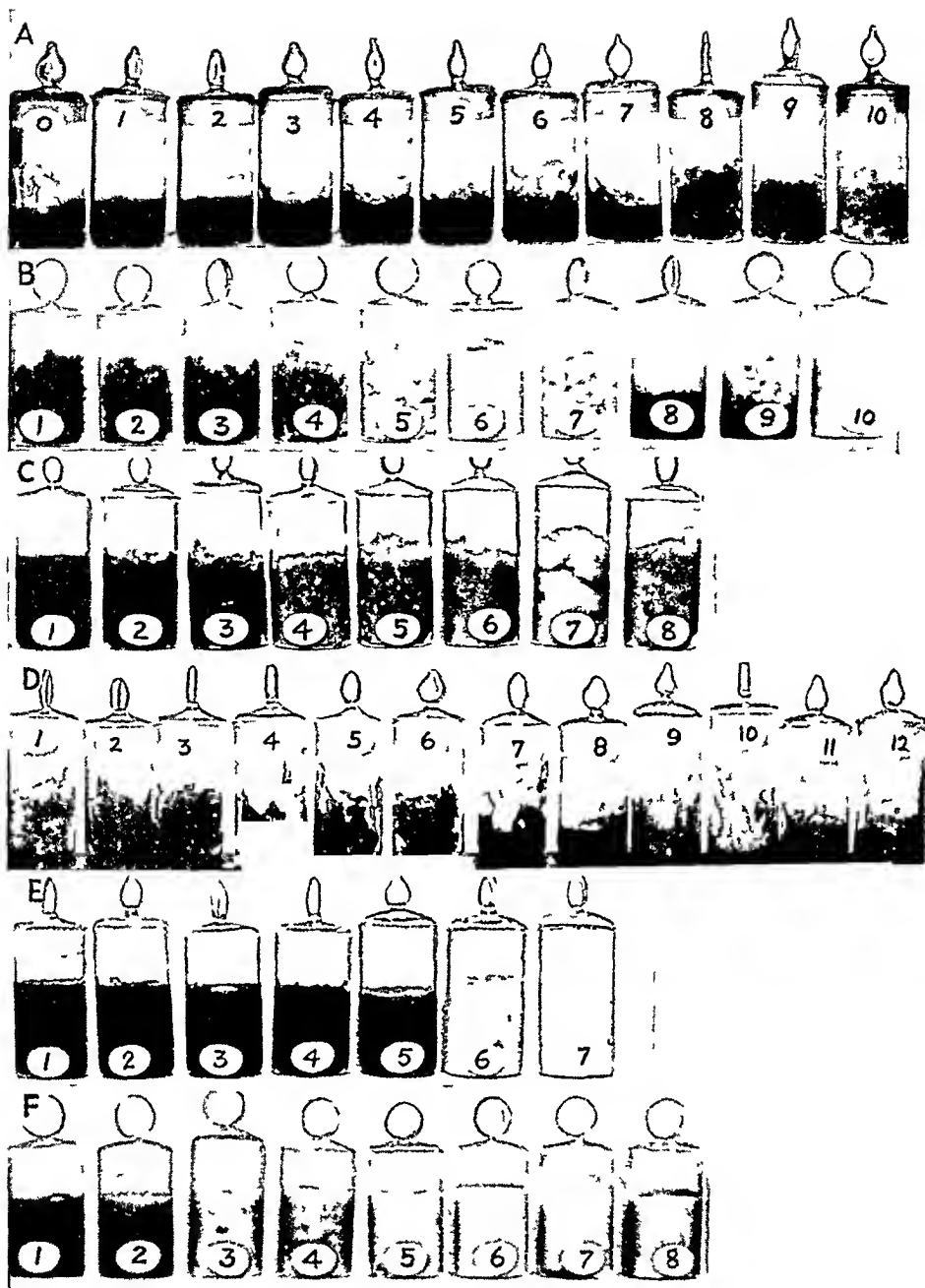
In the accompanying figure, *A* illustrates the point that acid fails to knock alkali off a basic proteinate, provided the mixture is concentrated and anhydrous. Vessel 0 contains a mixture of pure sodium caseinate and water made by adding to 50 Gm of a highly purified casein 20 cc of water and 80 cc of half-normal sodium hydroxide.⁴ The product is a faintly straw-colored transparent gel. The succeeding vessels contain an identical mixture, but there has been added to them strong hydrochloric acid in such quantity that the final mixture (vessel 10) contains just enough for complete neutralization of the base that was originally added to the casein. The intermediate tubes contain increasing amounts (in steps of 10 per cent) of the acid. It will be observed that over 70 per cent of the acid and not exceeding 80 per cent may be added before any change in the gel is perceptible.⁵ Beyond this point all the water is still bound, but the gels are drier and stiffer. When stirred they tend to fracture like crystals and so appear whiter. Their physical characteristics change, in other words, from those of egg white to those of a torn liver or kidney.

In the figure, *B* proves that only as free water is absent from the reaction mixture does the added acid join to the protein (and not to its contained alkali). Vessel 1 contains potassium caseinate (50 Gm of casein and 80 cc of half-normal potassium hydroxide) to which was added enough strong hydrochloric acid to neutralize completely the fixed

3 Such analyses may be found in Vierordt, Hermann. *Daten und Tabellen*, ed. 3, Jena, Gustav Fischer, 1906, p. 377.

4 This is the neutralization equivalent of casein according to T. B. Robertson.

5 Workers who wish to repeat these experiments need to remember that these colloid reactions take time. It requires about twenty-four hours for an acid or an alkali to combine with casein and to have the whole system come to equilibrium so far as the absorption of water is concerned. The same is true when acid is added to a basic caseinate, which should be done in fractions and slowly, with due care to the securing of an immediate and uniform mixture.



A, standard sodium caseinate to which increasing increments of hydrochloric acid have been added up to the point of complete neutralization of the base *B*, standard potassium caseinate to which increasing increments of water have been added and then hydrochloric acid to the point of complete neutralization Casein is definitely precipitated only in vessels 8, 9 and 10 *C*, standard potassium caseinate to which chemical equivalents of different acids (in the following order phosphoric, citric, acetic, lactic, hydrochloric, tartaric, hydrobromic and sulphuric acid) have been added *D*, standard sodium caseinate to which chemical equivalents of different fatty acids (of the acetic series and from formic through stearic) have been added *E*, a series of different basic caseinates to which chemical equivalents of phosphoric acid have been added *F*, a series of different acid caseinates (in the following order phosphate, citrate, acetate, lactate, chloride, tartrate, bromide and sulphate) to which chemical equivalents of potassium hydroxide have been added

base But no externally visible change has occurred in the gel The remaining vessels contain the same potassium caseinate (with its 80 cc of water), but before the necessary equivalent of acid was added, increasing amounts of water (in steps of 40 cc) were poured into them Failure of the acid to combine completely with the protein (combining instead with the potassium of the caseinate) is apparent in vessel 5, in other words, when the original amount of potassium caseinate has been mixed with 240 cc of water In percentage composition this represents progression from 60 per cent of water in vessel 1 to 83 per cent in vessel 5 Beyond this point (it should again be noted that it already lies well above the average value of the water content of all solid animal tissues) the action of the acid becomes increasingly that of neutralization of the fixed alkali in the caseinate (thus leading to a precipitation of the neutral casein and dehydration of the entire system) In vessel 10 (which contains 440 cc of water) separation of the neutral casein is complete

C shows that every acid acts like hydrochloric acid on a given basic caseinate Vessels 1 to 8 contain equal amounts of potassium caseinate (prepared by adding 200 cc of fifth-normal potassium hydroxide to 50 Gm of casein) There has been introduced into the successive vessels strong phosphoric, citric, acetic, lactic, hydrochloric, tartaric, hydrobromic and sulphuric acid in the order named and in an amount to neutralize completely the potassium of the system There is no difference in the several systems except as the first may be said to be the most homogeneous and the last, the least liquid "Precipitation" of neutral casein has not occurred anywhere

D in essence parallels C The vessels show that practically identical gels are obtained, so far as physical appearance is concerned, when the several members of the acetic series of fatty acids are added to the standard sodium caseinate prepared as in A Formic, acetic, propionic, butyric, valeric, caproic, caprylic, capric, lauric, myristic, palmitic and stearic acids have been added in chemically equivalent amounts to vessels 1 to 12 In vessels 10 to 12 the reaction mixtures were warmed

The findings described are of importance in connection with the problem of the absorption of fatty acid by any tissue or, specifically, by the intestinal tract The solubility of these acids in water falls almost to zero when valeric acid is passed To explain the absorbability of the higher members of the series, their combination with bile acids (to yield more "soluble" compounds) has recently been urged⁶ D shows that they are capable of direct absorption, through combination with protein, even up to stearic acid although as the series is ascended the process takes longer and may be less complete

6 Tashiro, Shiro Personal communication to the authors in 1935

The effects of adding a given acid (phosphoric) to a series of different basic caseinates (all prepared by mixing together 50 Gm of casein and 200 cc of water and the necessary weight of base to yield a fifth-normal mixture) is shown in *E*. The gels ammonium casein phosphate, potassium casein phosphate and so on through sodium, lithium and magnesium (vessels 1 to 5) require no comment. Those of calcium casein phosphate (vessel 6) and plumbic casein phosphate do require comment for while all continued to hold the water of their systems, they were less transparent. We take this to mark aggregation of the particles of protein into larger masses and as evidence of their lowered capacity for hydration. The situation has its parallel in the behavior of the corresponding metal soaps, though it remains a question in these experiments if, on allowing casein to stand with calcium hydroxide or lead oxide for several days, all the casein and base capable of combination have really reacted.

If the alkali and acid added to the reaction mixtures here described (containing about 80 per cent of water) are recalculated as their "salt" content, it amounts to 1.5 per cent. This is again a value of physiologic significance, for it lies well above the normal salt content of all fresh tissues or body fluids. The ash from spleen, for example, represents only 1.5 per cent, from brain, 1.41 per cent, from lung, 1.16 per cent, from intestinal canal, 1.07 per cent, from heart muscle, 1.06 per cent, from blood, 0.85 per cent, from kidneys, 0.8 per cent, and from skin, 0.7 per cent.⁷ But a protein hydrate that is first neutralized to its capacity with alkali shows no visible change on the addition of acid until more than 70 per cent of the neutralization value for the alkali is exceeded. This relation, too, is biologically significant because it is identical with the proportion of total base to total acid discoverable in the salts analyzed out of milk, blood plasma or any solid tissue. For instance, if the total base is taken as unity, the total acids of milk (with the phosphorus and sulphur of the protein and lipins included) constitute only 90 per cent of this value,⁷ for whole blood the value stands at 58 per cent,⁸ in the case of muscle, at 78 per cent.⁹

Being in essence linkages of amino-acids, it is not unnatural that proteins should combine with bases. But they are amphoteric and combine also with acids, even though the total combining value for them is lower (by about 30 per cent). Combination with acid also takes more time. We were interested in discovering whether acid caseinates (in the absence of free water) would combine with alkalis and so yield

7 Lincoln, Azariah Thomas, and Walton, James Henri. Quantitative Chemical Analysis, New York, The Macmillan Company, 1914, p. 88.

8 Vierordt,³ p. 200.

9 Vierordt,³ p. 426.

the triple compounds already described. Thus they do, as evidenced in *F*, even though the gels produced are not as homogeneous as when the reaction is carried out in the opposite direction. In all these vessels the acid caseinates (in the following order: casein phosphate, citrate, acetate, lactate, chloride tartrate, bromide and sulphate) were first made by adding to 50 Gm. of casein 200 cc. of the appropriate two-twenty-fifths-normal acid. (This amounts to only two fifths of the acid used for complete neutralization of the base when the basic caseinates were used as starting materials, purposely kept low in order not to exceed the neutralization capacity for acids of the casein itself.) After the addition of dry potassium hydroxide to the several acid caseinates (just sufficient to neutralize the acid) as the vessels show, no precipitation of the casein occurred.

We have also tried to produce these triple compounds directly by titrating dry salt into casein in the presence of minimal quantities of water or by allowing the pure casein to stand for long periods in contact with concentrated salt solution. In the instance of neutral salts (like sodium chloride) no change is noticeable in the hydration of the casein, but when salts that are definitely alkaline (like the potassium soaps) or acid (like the chlorides of weaker bases) are employed, partial union does take place, and (nonhomogeneous) gels are frequently obtained.

SUMMARY

After citation of some of the evidence which indicates that the "native" proteins are base-protein-acid compounds, a method is described which allows of their production artificially. The end is accomplished by working with reaction mixtures containing no free water and by adding to any protein (casein was used in the illustration) first a base and then an acid or vice versa. The allowable limit of water content for these systems in which alone such reaction was possible, was found to lie well above the normal water content of living tissue, while the amount of "salt" that could thus be bound to a protein, as well as the percentage relation of the base to the acid in such "salt," proved identical with biologic values.

MECHANISM OF PATHOLOGIC CALCIFICATION

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No attempt will be made to review the literature on various phases of calcification, because such reviews have been made recently by Ham,¹ Thomson and Collip,² Kay³ and Barr⁴. Several theories have been advanced in attempts to explain the mechanism of calcification of the soft tissues of the body. It is known that alkalinity favors the precipitation of calcium salts in vitro, and Hofmeister⁵ suggested that the calcification of the arteries might be due to the decreased carbon-dioxide tension of arterial blood with resulting increase in alkalinity. He also suggested that the tendency of the alveoli of the lungs, uriniferous tubules and gastric glands to calcify was due to the alkalinity of these structures brought about by the elimination of acids at these sites. Kleinmann⁶ found that dead tissues and necrotic areas were alkaline in reaction, and he attributed their tendency to calcify to this fact. It is recognized that fatty degeneration frequently precedes calcification of the soft tissues, and according to Klotz⁷ the formation of calcium soaps, which are subsequently transformed into calcium phosphate and calcium carbonate, constitutes the mechanism of calcification. Wells⁸ has presented evidence which would seem to indicate that the theory of Klotz regarding calcification is untenable. According to Robison,⁹ normal ossification is brought about by the enzyme phosphatase. It is recognized that the presence of vitamin D is necessary for normal ossifi-

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1 Ham, A W Arch Path **14** 613, 1932

2 Thomson, D L, and Collip, J B Physiol Rev **12** 309, 1932

3 Kay, H D Physiol Rev **12** 384, 1932

4 Barr, D P Physiol Rev **12** 593, 1932

5 Hofmeister, F Ergebn d Physiol **10** 429, 1910

6 Kleinmann, H Biochem Ztschr **196** 161, 1928

7 Klotz, O J Exper Med **7** 633, 1905

8 Wells, H G A Survey of the Problem, in Cowdry, E V Arterio-sclerosis, New York, The Macmillan Company, 1933

9 Robison, R Biochem J **17** 286, 1923

cation, and evidence has been presented to show that large doses of viosterol, as well as of the internal secretion parathormone, may bring about calcification of the soft tissues¹⁰ It is known that the deposition of calcium salts begins in the intima of the arteries, and there is considerable evidence that this is initiated by injury resulting, for example, from an infection such as syphilis, the injection of certain poisonous chemicals, the stress and strain of modern life or excessive physical work

Active, injured and dying tissues are electronegative to inactive, uninjured and sound tissues The injured end of a muscle, for instance, is electronegative to the sound surface, and the contracted portion of a muscle is electronegative to the relaxed portion The contracted part of the heart is electronegative to the uncontracted part, and this fact renders the making of the electrocardiogram possible It was while studying these differences of potential in the animal and measuring the action and demarcation currents that it occurred to us that herein might be found an explanation for calcification of the soft tissues, and the following investigation was accordingly carried out

EXPERIMENTS

With the use of ammonium molybdate paper the injured or cut ends of gastrocnemius muscles of frogs were tested for phosphate, and it was found to be present in definitely larger quantities on the cut end than on the uninjured surfaces This suggested to us that the electronegativity of the injured portions of these muscles might be caused by the negatively charged phosphate ions, and the following experiments were carried out to determine whether this was true

The gastrocnemius muscles of frogs were removed, skinned and cut transversely near one end The cut end was placed against one non-polarizable boot electrode and the sound surface against the other electrode, as shown in the insert in figure 1 Both boot electrodes were kept moist with a physiologic solution of sodium chloride By means of wires a delicate micro-ammeter was connected with the boot electrodes and the demarcation current measured in micro-amperes For the muscles of these medium-sized frogs the demarcation current was found to be of the magnitude of from 2 to 4 micro-amperes It was found that the application of a solution of calcium chloride, as well as of barium chloride to the cut end of the muscle caused the disappearance of the demarcation current, and the application of a weak solution of phosphoric acid or of disodium phosphate restored the current to its original value It was also found that if a strip of filter paper or a piece of wire was placed across the electrodes instead of the muscle and if one end of the filter paper or wire was moistened with a solution of phosphoric acid or disodium phosphate and the other end with a

10 Hueper, W Arch Path 3 14, 1927

physiologic solution of sodium chloride, a current was set up similar to the demarcation current of the injured muscle and of about the same magnitude. This current passed from the phosphate to the chloride end of the paper or wire. The application of calcium chloride to the end of the filter paper or wire that was moistened with the phosphate decreased the current in the same way that it decreased the demarcation current when applied to the cut end of the muscle. The current could be restored by moistening the end of the filter paper or wire with phosphoric acid, as had been found to be the case with the muscle.

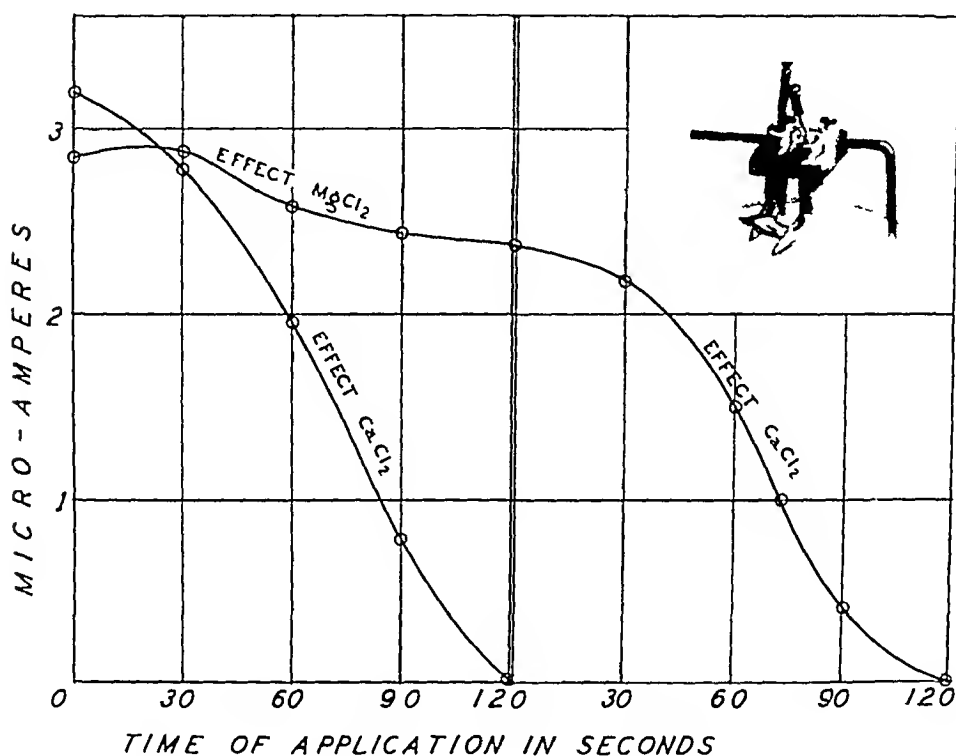


Fig 1—The insert shows the injured or electronegative end of a muscle against one boot electrode and the sound or electropositive surface against the other electrode. The curves show that the application of calcium chloride to the injured end of the muscle does away with the demarcation current, while magnesium chloride has only a little effect.

In figure 1 are given curves showing the quantitative effect of application of a twice normal solution of calcium chloride and magnesium chloride on the demarcation current of injured gastrocnemius muscles of frogs. The method of applying the calcium chloride and magnesium chloride was to suspend the muscle and permit its cut end to touch the surface of the solution for thirty second intervals and measure the demarcation current after each application. It will be seen in figure 1 in the curve for the effect of calcium chloride that the demarcation current of the muscle previous to the application of calcium chloride was

32 micro-amperes, after thirty seconds of treatment with calcium chloride it was reduced to 28 micro-amperes, after the second thirty second application or after sixty seconds of treatment with calcium chloride to 2 micro-amperes, and after ninety seconds, to 0.75 micro-amperes. After one hundred and twenty seconds the current had disappeared. It may also be seen in figure 1 in the curve for the effect of magnesium chloride that a twice normal solution of magnesium chloride had little effect on the demarcation current. The curve for the effect of magnesium chloride and of calcium chloride shows that after magnesium chloride had been applied to the cut end of the muscle and had produced practically no effect, the application of calcium chloride to the cut end of the same muscle promptly decreased the current and caused it to disappear. It should be mentioned in this connection that barium chloride was found to be as effective as calcium chloride in causing the disappearance of the demarcation current. It is assumed that the positively charged calcium and barium ions did away with the demarcation current by combining with the negatively charged phosphate ions at the cut end of the muscle to form insoluble and non-ionized barium phosphate and calcium phosphate, whereas the application of magnesium chloride formed the more soluble and ionized magnesium phosphate. The preceding observations have been repeated time and again by us, and the experiments have been used by students as a part of routine work in the laboratory.

It is also known that the contracted portion of a muscle is electro-negative to the relaxed part, and the observations made on the injured muscle suggest that this electronegativity is probably due also to the negatively charged phosphate ions arising from the hydrolysis of creatine phosphate and adenylypyrophosphate in the contracted part of the muscle. Since massive doses of viosterol and parathormone produce calcification of the soft tissues, the effect of these two substances on the demarcation current was also studied in a manner similar to the preceding, and it was found that the application of these substances to the cut end of the muscle had no effect on the demarcation current.

The demarcation current of the arteries of anesthetized dogs was measured. This was done with the use of a micro-ammeter and platinum electrodes. It was found that when two uninjured parts of the carotid artery were connected with the micro-ammeter, practically no current flowed, but when an injured portion, either of the exterior or interior of the artery, was connected to an uninjured part a current flowed from the uninjured to the injured surface, just as was found to be the case with the gastrocnemius muscles. The electronegativity of the injured intima of the artery is attributed to the negatively charged phosphate ions as in the injured end of the gastrocnemius muscles and the sub-

sequent calcification of the intima is attributed to the combination of the positively charged calcium ions of the blood with the negatively charged phosphate ions of the injured intima to precipitate the insoluble calcium phosphate and form the plaque

With advance in age calcium salts are gradually deposited also in the crystalline lens of the eye, resulting in a receding of the near point of distinct vision and loss in the power of accommodation. At 20 years of age a normal person possesses 10 diopters of accommodation, but at the age of 60 all this has been lost, and this loss has occurred at the uniform rate of $\frac{1}{4}$ diopter per year. So uniformly and constantly does this loss in the power of accommodation occur that the age of a person may be ascertained with a high degree of accuracy by determining the near point of distinct vision. With the accumulation of very large amounts of calcium salts in the crystalline lens of the eye, cataract results.¹¹

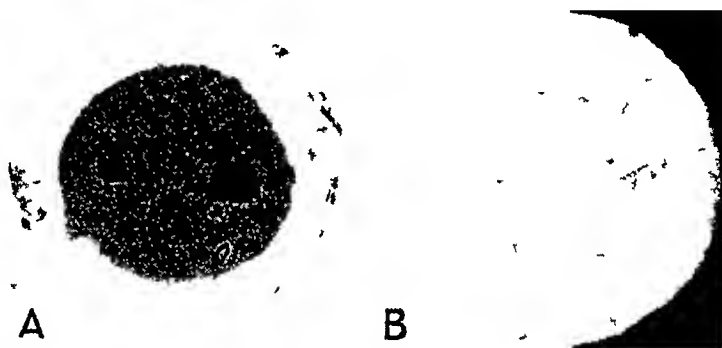


Fig 2—Lens 1 (*A*) was immersed in twice normal potassium chloride and lens 2 (*B*) in twice normal calcium chloride. The potassium chloride produced nuclear opacity, leaving the cortex transparent, while the calcium chloride produced cortical opacity without affecting the nucleus.

In figure 2 are shown fresh crystalline ox lenses that had been immersed in twice normal solutions of potassium chloride and calcium chloride. Lens 1 (*A*) was immersed in potassium chloride and lens 2 (*B*) in calcium chloride. It will be seen that potassium chloride produced an opacity of the nucleus of the lens without affecting the transparency of the cortex, and that the calcium produced an opacity of the cortex without affecting the nucleus. This observation suggests that calcium salts may play a rôle in the production of cortical cataract and potassium salts in that of nuclear cataract. It is known that the crystalline lens grows throughout life and that this growth, like that of a cabbage, takes place from the interior. So the oldest portion of the

¹¹ Burge, W. E. *Arch Ophth* 38:447, 1909.

lens is the cortex and the youngest the nucleus. Hence, there is a tendency for the older portion of the crystalline lens, the cortex, to combine with calcium salts, comparable with the tendency of the arteries to become calcified with advance in age.

The tender green branches of several different kinds of greenhouse plants were tested to determine whether there was a demarcation current or current of injury in plants similar to that in animals. This was done by placing a tender green branch across the boot electrodes shown in the insert in figure 1, with the cut end of the branch against one electrode and the uninjured surface against the other. When this was done it was found that a current flowed from the uninjured surface of the branch to the injured end, similar to that of the muscle, but the strength of the current was much less than in the muscle. The application of a weak solution of calcium chloride to the injured end of the stem of the plant did away with the demarcation current, just as was found to be the case with the muscle. The inorganic salt deposited in the arteries of animals as they grow older is principally calcium phosphate, whereas calcium oxalate is the salt deposited in plants as they grow older. It is assumed that the negatively charged oxalate ions are responsible for the electronegativity of the injured portion of the plant, just as the negatively charged phosphate ions are responsible for the electronegativity of the injured portion of the muscle. The positively charged calcium ions combine with the negatively charged phosphate ions of the injured muscle and with the negatively charged oxalate ions of the injured plant to precipitate calcium phosphate and calcium oxalate, respectively, and in this way do away with the current of injury or demarcation current and form the calcareous deposit.

SUMMARY

With the use of a micro-ammeter the demarcation current of injured frogs' muscles was measured and found to be of the order of magnitude of from 2 to 4 micro-amperes. A demarcation current in injured branches of greenhouse plants was also observed, but this was much less than in the animal muscles.

The presence of phosphate on the injured portion of the muscle was shown by the use of ammonium molybdate paper. The application of a twice normal solution of calcium chloride or barium chloride to the injured portion of the muscle or of the plant did away with the demarcation current, and a weak solution of phosphoric acid or disodium phosphate restored it.

The electronegativity of the injured portion of a muscle is attributed to the negatively charged phosphate ions, and the disappearance of the current on the application of calcium chloride and barium chloride, to the

combination of the positively charged calcium and barium ions with the negatively charged phosphate ions, to precipitate insoluble calcium and barium phosphate

The electronegativity of the contracted portion of a muscle is also attributed to the negatively charged phosphate ions, arising probably from the hydrolysis and ionization of creatine phosphate and adenylypyrophosphate in the contracted part of the muscle

Injury to the intima of the carotid artery of dogs renders the injured portion electronegative to the uninjured portion, and calcification of arteries may result from the combination of the positively charged calcium ions of the blood with the negatively charged phosphate ions at the site of the injured intima to precipitate calcium phosphate

Potassium chloride produced an opacity of the nucleus of the crystalline lens without affecting the transparency of the cortex, while calcium chloride produced an opacity of the cortex without affecting the nucleus. This observation suggested that calcium salts may play a rôle in the production of cortical cataract, and potassium salts in the production of nuclear cataract

SUSCEPTIBILITY TO DENTAL CARIES IN THE RAT

V INFLUENCE OF CALCIUM, PHOSPHORUS, VITAMIN D AND CORN OIL

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AND

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In previous reports¹ we have described the production in rats of experimental dental caries and indicated some of the conditions that give rise to the disease or modify its course. We have shown (*a*) that the lesions in question, identified microscopically in all instances, closely resemble naturally occurring dental caries in man and differ in important respects from lesions described as "macroscopic caries" by many other workers, (*b*) that experimental caries results from feeding diets the major constituent of which is coarsely ground rice or corn, and that the disease does not occur when the cereal is very finely ground, (*c*) that experimental caries may be produced either with diets deficient in minerals, vitamin D and protein or with diets adequate in all nutritional respects, but in the latter case the incidence of experimental caries is lower than in the former, and (*d*) that the relative protective effect against experimental caries produced by adequate diets appears to depend on some one calcifying food or some combination of the calcifying foods, but the precise agency involved could not be determined from the data.

EXPERIMENTS

The experiments reported here were an attempt to define more exactly the food agent or agents responsible for the reduction of experimental caries observed in rats that received diets with adequate calcifying properties. Two series were studied, for each of which the basis of reference was the deficient diet of rice passed through a sieve having 10 meshes per linear inch (2.54 cm), dextrin and spinach, which was previously employed, to which additions were made individually. The diets used are given in table 1.

In series I, diets 55 and 56 contain calcium and phosphorus in relatively high levels with an adequate ratio (1.5) without cod liver oil and with 2 per cent of cod liver oil. Diet 57 is the basal control. Diet 58 contains rice passed through a 100-mesh sieve in place of that passed through a 10-mesh sieve. In diets 60

This study was conducted in part with the aid of a grant from the Commonwealth Fund for research in dental caries.

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1 (*a*) Rosebury, T., Karshan, M., and Foley, G. *J. Dent. Research* **12** 464, 1932, (*b*) *ibid* **13** 379, 1933, (*c*) *J. Am. Dent. A.* **21** 1599, 1934.

and 61 calcium is added to make a high calcium-phosphorus ratio (6.9), the latter diet again contains rice passed through a 100-mesh sieve. Diet 63 contains calcium and phosphorus added to overcome the gross deficiency of the basal diet but leaving the ratio low (0.4). Diet 64 contains 7 per cent of wheat gluten as the only addition to the basal formula. In series II, diet 65 is the basal control, and each of the experimental diets is supplemented by a single item. Diets 66 and 67 contain, respectively, 2 and 5 per cent of a dilution of viosterol (250 D) in corn oil adjusted to have the vitamin D potency of cod liver oil,² diets 68 and 69, 2 and 5 per cent of cod liver oil, respectively, and diets 70 and 71, 2 and 5 per cent of corn oil, respectively. Diet 72 is the same as the control, but animals in this group were irradiated with an air-cooled quartz mercury vapor arc lamp in

TABLE 1—Experimental Diets

	Percentage									
	Brown Rice, 10 Mesh	Brown Rice, 100 Mesh†	White Potato Dextrin	Calcium Car- bonate	Potassium Phos- phate (KH ₂ PO ₄)	Cod Liver Oil (Mead's)	Viosterol in Corn Oil‡	Corn Oil (Marzola)	Wheat Gluten	Spinach Leaves#
Series I										
Diet 55	81		8	6	5					2.46
Diet 56	79		8	6	5	2				2.43
Diet 57*	92		8							0.03
Diet 58		92	8							0.03
Diet 60	88		8	4						1.63
Diet 61		88	8	4						1.63
Diet 63	88		8	1	3					0.43
Diet 64	85		8						7	0.03
Series II										
Diet 65*	92		8							0.03
Diet 66	90		8				2			0.03
Diet 67	87		8				5			0.03
Diet 68	90		8							0.03
Diet 69	87		8			2				0.03
Diet 70	90		8			5				0.03
Diet 71	87		8					2		0.03
Diet 72	92		8					5		0.03

* Control
† Ground repeatedly until the entire product passed through the 100 mesh sieve
‡ Viosterol 250 D (Mead's), 0.4 per cent in corn oil (dilution = 1D)
§ See Rosebury, Karshan and Foley,^{1c} table 8
5 Gm per rat per day in all
|| Rats in this group were irradiated with a quartz mercury vapor arc lamp for twenty minutes at 2 feet (60.96 cm) three times each week, the lamp was run fifteen minutes before the animals were placed under it, and food and water were removed from the cage during irradiation

dosage sufficient, as will be noted later, to supply adequate vitamin D.³ All vitamin D preparations were added to the dry diets freshly twice each week. Diets and water were fed ad libitum.

2 Since this paper was submitted for publication it has come to our attention that viosterol 250 D has one hundred instead of two hundred and fifty times as much vitamin D as the cod liver oil used in these experiments (Bills, C. E. *Physiol. Rev.* **15**, 1, 1935, personal communication to the authors). On this basis the vitamin D content of diet 66 is equivalent to 0.8 instead of 2 per cent of cod liver oil, and that of diet 67 is equivalent to 2 instead of 5 per cent of cod liver oil.

3 The Hanovia Manufacturing Company furnished the lamp used in these experiments.

As in previous experiments, rats were bred on our modified McCollum stock ration and distributed among the experimental groups twenty-two days after birth. Litter-mates were distributed separately in the two series. Each diet group contained from 10 to 13 animals. The period of experimental feeding ranged from forty-five to one hundred and eighty days, at intervals during this period animals were removed from the experimental groups in lots of litter-mates, bled by cardiac puncture for analyses of calcium and phosphorus and killed with chloroform. One side of the mandible of each animal was used for the preparation of decalcified (celloidin) sections stained with hematoxylin and eosin. Generally the side that showed the fewer macroscopic lesions was selected for this purpose. The analytic methods employed were those described in the previous report in this series,^{1c} in which the method used for identification and rating of lesions of caries was also described in full. Lesions are rated according to size and degree of penetration from 1 to 10. The "index" value, as applied in this and the previous report, is obtained by dividing the total caries score for the group by the number of animals in the group. This value provides a satisfactory measure of the incidence of caries in the experimental groups, as indicated by its relatively close reproducibility. Lesions identified as resulting from fracture of a cusp, including those referred to by other workers as macroscopic caries, were recorded in these studies as such, but are not included in the tables, which deal only with fissure caries.

RESULTS

Table 2 presents data for the individual groups on the incidence of caries, calcification of teeth and bone and blood calcium and phosphorus. The values for caries are given in terms of both the percentage of animals affected in the group and indexes. The two sets of values are not well correlated, since only the index values reflect the number and size of the lesions as well as their distribution by animals. Data on calcification, as determined by examination of the sections, are given here roughly only. All the pathologic changes observed have been previously described by ourselves and other investigators. It will be noted that the occurrence and kind of defects of calcification were those to be expected from the character of the respective diets. The low calcium diets resulted in uniformly defective dentin, whereas the high calcium diets produced local defects in calcification, generally over the pulpal floor and in the roots of the molars. Diets 66 to 69, which contained vitamin-oil preparations as the only additions to a basal diet very low in minerals, produced molar dentin that was slightly defective locally and alveolar bone normal in quality but apparently reduced in amount. The same was true of the group that received ultraviolet irradiation (72). The blood values likewise parallel closely the histologic observations and are in the expected range for the several diets. It is interesting that the blood values for the two diets containing rice passed through a 100-mesh sieve (58 and 61) both indicate somewhat more severe deficiency than the respective diets with rice passed through a 10-mesh sieve. Growth was recorded as a check on the condition of the animals during the course of experimental feeding, but the method employed

of removing animals from each group at intervals during the progress of the experiment produces average curves that are not strictly comparable. Hence curves for growth are not presented.

In order to assess the significance of the reduced caries indexes of the experimental groups, as compared with the control groups, the index values have been treated statistically.⁴ As a basis for this treatment all groups fed the control diet (with rice passed through a 10-mesh sieve, 92 per cent, dextrin, 8 per cent, plus from 3 to 5 Gm of spinach per rat per day and water ad libitum) since these experiments

TABLE 2—Data on Incidence of Caries, Calcification and Blood Calcium and Phosphorus Values in Diets 55 to 72*

Diet Group	Number of Animals	Additions to Basal Diet	Experimental Period, Days	Calcification		Caries		Blood Calcium, Mg per 100 Cc	Blood Phosphorus, Mg per 100 Cc
				Teeth	Bone	Percent age of Animals	Index		
55	12	High calcium, high phosphorus	45 177	N	N	67	2.9±1.0	10.90±0.37	6.04±0.16
56	12	Diet 55 + 2 per cent CLO	45 177	N	N	50	2.3±1.0	10.75±0.13	7.16±0.31
57	12	None	45 120	VD	VD	75	6.3±2.4	5.52±0.32	8.30±0.78
58	10	None (100 mesh)	45 120	VD	VD	0	0	5.13±0.35	8.75±0.40
60	10	High calcium	44 105	LD	VD	90	4.5±1.6	9.55±0.54	3.92±0.29
61	10	High calcium (100 mesh)	44 115	LD	VD	0	0	11.15±0.33	3.68±0.31
63	11	Low calcium	45 170	D	D	45	3.2±1.6	6.47±0.16	6.90±0.25
64	11	7 per cent protein	45 105	VD	VD	82	5.4±1.3	5.47±0.20	7.56±0.25
65	12	None	55 181	VD	VD	100	7.8±1.5	5.62±0.30	8.08±0.36
66	12	Vios in CO, 2 per cent	55 181	LSD	NR	33	2.1±1.1	10.16±0.41	6.23±0.16
67	13	Vios in CO, 5 per cent	47 181	LSD	NR	31	0.9±0.5	10.38±0.30	7.67±0.22
68	10	2 per cent CLO	47 181	LSD	NR	50	2.3±1.3	10.75±0.12	7.58±0.34
69	13	5 per cent CLO	47 181	LSD	NR	46	3.1±1.5	10.30±0.23	7.43±0.50
70	11	2 per cent CO	49 181	VD	VD	82	3.0±0.9	6.22±0.24	8.26±0.67
71	12	5 per cent CO	47 181	VD	VD	50	2.8±1.3	5.49±0.17	7.13±0.52
72	12	Irradiated	55 181	LSD	NR	58	4.5±1.4	10.61±0.20	7.26±0.28

* In this and other tables the following abbreviations are used: CLO, cod liver oil, CO, corn oil, Vios, viosterol, N, normal, D, defective, VD, very defective, LD, locally defective, LSD, locally slightly defective, NR, normal in quality but reduced in amount. The ± values are standard deviations.

were started in 1931, with one exception, were pooled to form a group of 94 animals. The exception was the group on diet 24 (1934), which was omitted as clearly aberrant. This control group was then pooled individually with each experimental group to be compared to obtain a mean index (m_0) and its standard deviation. The standard deviation of the difference between the index of the experimental group and

4 Prof. Earle B. Phelps of the Department of Public Health of the College of Physicians and Surgeons collaborated in formulating the statistical method used. The method is based on the assumption of homogeneity of the different groups pooled to obtain a mean index, parts of the pooled group being then compared with the whole to test this assumption.

the mean index was derived as the square root of the sum of the squared standard deviations of the two means. The ratios of these differences to their standard deviations were interpreted from the table given by Pearl.⁵ The following equations were employed

$$\text{Standard deviation of an index } (\sigma_m) = \sqrt{\frac{\sum d^2}{N(N-1)}}$$

$$\text{Standard deviation of a difference } (\sigma_D) = \sqrt{\sigma_o^2 + \sigma_m^2}$$

$$\text{Mean of a series of (two or more) component means } (m_o) = \frac{\sum mf}{N}$$

$$\text{Standard deviation of } m_o (\sigma_o) = \sqrt{\frac{\sum (\sum d^2 + [f(m_1 - m_o)^2])}{N(N-1)}}$$

TABLE 3—Significance of Reductions in Caries Produced on Addition of Calcifying Foods, Corn Oil or Protein to the Basal Deficient 10-Mesh Rice-Dextrin-Spmach Diet

Diet Group	Additions to Basal Diet	Number of Animals	Calcification, Blood Calcium and Phosphorus	Caries Index, m	m_o	$m_o - m = D$	$\frac{D}{\sigma_D}$	Odds Against Random Occurrence of Difference
Pooled control	None	94	D*	7.6±0.6				
55	High calcium, high phosphorus	12	N	2.9±1.0	7.1±0.6	4.2±1.2	3.5	2,149 to 1
56	Diet 55 + 2 per cent CLO	12	N	2.3±1.0	7.0±0.6	4.7±1.2	3.9	10,390 to 1
60	High calcium	10	D	4.5±1.3	7.3±0.5	2.8±1.4	2.0	21 to 1
63	Low calcium	11	D	3.2±1.6	7.1±0.5	3.9±1.7	2.3	46 to 1
64	7 per cent protein	11	D	5.4±1.3	7.4±0.5	2.0±1.4	1.4	5 to 1
66	Vios in CO, 2 per cent	12	N	2.1±1.1	7.0±0.5	4.9±1.2	4.1	30,000 to 1
67	Vios in CO, 5 per cent	13	N	0.9±0.5	6.8±0.5	5.9±0.7	8.4	∞ to 1
68	2 per cent CLO	10	N	2.3±1.3	7.1±0.5	4.8±1.4	3.4	1,483 to 1
69	5 per cent CLO	13	N	3.1±1.5	7.1±0.5	4.0±1.6	2.5	80 to 1
70	2 per cent CO	11	D	3.0±0.9	7.1±0.5	4.1±1.1	3.7	4,637 to 1
71	5 per cent CO	12	D	2.8±1.3	7.1±0.5	4.3±1.4	3.1	516 to 1
72	None, irradiated	12	N	4.5±1.5	7.2±0.5	2.7±1.6	1.7	10 to 1
25, 66, 67, 68, 69	Vios in CO or CLO	55	N	1.9±0.5	5.5±0.5	3.6±0.7	5.1	4,000,000 to 1
27, 28, 37, 38, 56	Normal calcium and phosphorus + 2 or 5 per cent CLO	38	N	1.6±0.5	5.9±0.5	4.3±0.7	6.1	700,000,000 to 1
70, 71	2 or 5 per cent CO	23	D	2.9±0.8	6.7±0.5	3.8±0.9	4.2	50,000 to 1

* See footnote, table 2

In these equations σ indicates the standard deviation, D , the difference (between two means), d , the deviation (of an individual value from the mean), N , the number of animals in the whole group considered, f , the number of animals in a component group, and m , the mean (or index)

The results of this analysis are given in table 3, in which data on calcification and dietary composition are repeated to facilitate comparisons. The reduction of caries obtained with diets 55 and 56, containing

5 Pearl, R. Introduction to Medical Biometry and Statistics, Philadelphia, W. B. Saunders Company, 1930

calcium and phosphorus in high levels and a normal ratio, without cod liver oil and with 2 per cent of cod liver oil, respectively, are clearly significant, while those obtained with diets 60, 63 and 64 are not. The results obtained with the diets containing rice passed through a 100-mesh sieve (58 and 61) are not included in the table. Outright prevention of caries was obtained with both these diets. The group of diets in which vitamin D in oil was present either as viosterol or in cod liver oil produced reductions the odds against the insignificance of which range from 80 to 1 to a value approaching infinity to 1, and it is interesting that the extremes of this range both fall in the groups that received the higher percentage of the vitamin-oil preparations. The results obtained with diets 70 and 71, which were set up as controls for the inert oil in diets 66 to 69, are surprising, since a clearly significant reduction of caries was obtained with each.

Since the standard deviation decreases as the number of animals in the group is increased, small differences become more significant as the size of the group is increased. This fact is apparent in the comparisons for the pooled groups given in the lower part of table 3. The mean difference between the control group and the groups that received vitamin D-oil preparations as the sole additions to the basal diet (diets 66 to 69, plus diet 25—containing 2 per cent of cod liver oil—from an earlier series) is clearly significant. Similarly, when group 56 is pooled with previous groups that received diets containing normal calcium and phosphorus plus 2 or 5 per cent of cod liver oil (27, 28, 37 and 38) and when the two groups fed the corn oil diet (70 and 71) are pooled, the mean differences are clearly significant.

It is of further interest to determine whether the groups that received calcifying foods, corn oil or ultraviolet irradiation differ significantly with respect to each other. To determine this, the entire series of 147 animals, including groups on diets 25, 26, 27, 28, 37 and 38, in addition to the diets described here which yielded significantly reduced indexes of caries as compared with the control, were pooled to derive a mean index and standard deviation. When the indexes of the component groups are compared with the index of the complete series by the method previously used, it becomes clear that little weight can be attached to differences between these groups. This analysis is shown in table 4. It is interesting that the reduction of caries obtained with diets 70 and 71, although less in degree than the mean reduction obtained with the adequately calcifying diets, nevertheless falls within this observed variation. It is also noteworthy that in group 72 (irradiated animals) the result does not differ significantly from this complete series or from the control group (table 3).

To determine whether the blood values for calcium and phosphorus are related to the incidence or degree of caries a similar method of

analysis was used. In this instance, however, it is obvious that the abnormal values of animals that received deficient diets could not be used in the analysis, for although they would appear to be directly related to the caries indexes the fact that similar abnormal values were obtained with the diets containing rice passed through a 100-mesh sieve in the complete absence of caries clearly indicates that such abnormal values do not necessarily imply the occurrence of caries. It seemed valid rather to determine whether the variation in the blood values for calcium and phosphorus among the groups in which normal averages were obtained are related significantly to the degree of caries shown by those animals individually. Thus, 86 animals for which analytic data are available were treated as a unit as before, and mean values and standard deviations derived for calcium and phosphorus. This group

TABLE 4—*Comparative Influence of Calcium, Phosphorus, Vitamin D and Corn Oil on Experimental Dental Caries in Rats, Significance of Differences Between Experimental Groups*

Diet Group	Additions to Basal Diet	Number of Animals	Average Calcium to Phosphorus Ratio	Calcification, Blood Calcium and Phosphorus	Caries Index	$\frac{D}{\sigma D}$	Odds Against Random Occurrence of Difference
Complete series		147			2.5 ± 0.3		
25, 66, 67, 68, 69	Vios in CO or CLO	55	0.12	N*	1.9 ± 0.5	0.9	2 to 1
27, 28, 37, 38, 56	Normal calcium and phosphorus + 2 or 5 per cent CLO	38	1.31	N	1.6 ± 0.5	1.3	4 to 1
70, 71	2 or 5 per cent CO	23	0.12	D	2.9 ± 0.8	0.4	Less than 1 to 1
72	None, irradiated	12	0.12	N	4.5 ± 1.4	1.1	3 to 1

* See footnote, table 2

was then subdivided, without regard to experimental diets, into those with no caries and those with caries equivalent to a score from 1 to 3, 4 to 6 and 7 or more, respectively. When these groups are compared with the complete series, with the exception of calcium in the group with the greatest incidence of caries, the differences are less than their standard deviations, and it is clear that none is significant.

COMMENT

The result obtained with diet 58 confirms our previous finding that caries is prevented outright in the low calcium diet when the rice is ground to pass through a 100-mesh sieve. The absence of caries with diet 61 indicates that the same is true in the case of a high calcium diet. It is to be noted that each of these diets was not less severe in nutritional character than the corresponding diet containing rice passed through a 10-mesh sieve. It is also noteworthy that the high calcium

diet containing rice passed through a 10-mesh sieve (60) produced less caries than the corresponding low calcium diet, and although the difference is not significant it is clear that a relative deficiency of phosphorus is not more productive of caries than a deficiency of calcium. Addition of calcium and phosphorus to the basal diet, such as to produce a slight improvement in ratio but leaving the diet (63) relatively low in calcium, also resulted in a decrease of caries of doubtful significance. Addition to the basal diet of 7 per cent of protein in the form of wheat gluten (diet 64) did not produce a significant change in the incidence of caries, suggesting that the protein deficiency in the basal diet is not a factor in the production of the lesions.

The findings as a whole, particularly with the corn oil and more adequately calcifying diets, may be considered as they bear on three aspects of the problem: the comparative influence of the calcifying

TABLE 5—*Relationship of Blood Calcium and Phosphorus to Experimental Dental Caries in Rats. Significance of Differences Between Experimental Groups Showing Generally Normal Values*

Diet Group	Num ber of Ani mals	Calcium			Phosphorus		
		Mean	D σ _D	Odds Against Random Occurrence of Difference	Mean	D σ _D	Odds Against Random Occurrence of Difference
Complete series	86	10.56±0.08			7.22±0.12		
Caries score = 0	50	10.49±0.09	0.6	1 to 1	7.25±0.17	0.2	Less than 1 to 1
Caries score = 1-3	12	10.37±0.21	0.9	2 to 1	7.22±0.31	0.0	0
Caries score = 4-6	10	10.43±0.18	0.7	1 to 1	7.24±0.29	0.1	Less than 1 to 1
Caries score = 7 or more	14	11.02±0.28	1.6	8 to 1	7.00±0.31	0.7	1 to 1

elements, vitamin D and oil, the influence of varying amounts of these substances, and the mechanism whereby they act to reduce the incidence of caries.

The finding that corn oil, supplementing a diet very deficient in minerals and without vitamin D, produced a reduction of caries statistically equivalent to that produced by adequately calcifying diets must lead one to doubt the importance of vitamin D in this connection. Likewise, the effect on caries observed on ultraviolet irradiation, although inconclusive, helps to sustain the implication that the fat content of the vitamin D preparations rather than their vitamin content is the more potent protective agent against caries. The fact that calcium and phosphorus in high levels and a normal ratio in the absence of vitamin D or oil (diet 55) produced a significant reduction in caries, on the other hand, prevents exclusion of the calcifying elements as participants in this protective effect.

In considering the bearing of our data on the quantitative aspects of the caries-reduction phenomenon, it may be important to emphasize

that all the evidence obtained points to the coarse cereal particles as the essential caries-producing agents. When the basal coarse rice diet is supplemented with various foods, whether or not they improve its nutritional quality, the result is not more than partial reduction of caries, outright prevention regularly occurs only when the coarse cereal is ground to a flour. It is clear from these experiments that this relative protective effect may be contributed either by vitamin D-oil preparations, by calcium and phosphorus in normal ratio, by corn oil or by calcium and phosphorus in normal ratio with vitamin D in oil. The last-named combination produced the greatest average reduction in caries (table 4), but the statistical analysis indicates that none of these agencies can be regarded as superior to the others. The results obtained with diets 66 to 69, particularly, suggest that 5 per cent of the vitamin D-oil preparations is not necessarily more effective than 2 per cent, and the result in the case of the single diet 56 further suggests that even in the presence of what may be regarded as optimal dietary conditions—calcium and phosphorus in high levels and a normal ratio, with 2 per cent of cod liver oil—the protective effect cannot be depended on to exceed that produced by 2 per cent of cod liver oil or its viosterol or fat equivalent alone.

We have pointed out before that although adequately calcifying diets tend, in general, to produce less caries than comparable deficient diets, the difference in caries cannot be directly related to corresponding differences in the picture of calcification. The data recorded here confirm this point of view and make it clear as well that variations in blood calcium and phosphorus, within a generally normal range, do not reflect variations in the incidence of caries in individual animals. The mechanism underlying these facts is not clear. If the results of a given deficient caries-producing diet are compared with those obtained with a similar diet in which the deficiencies are corrected, the caries values in the two groups will appear to be related to these differences, to the degree of calcification determined histologically and to the blood values of calcium and phosphorus. Yet the results in this study of the experiments with rice passed through the 100-mesh sieve, incorporating both types of calcifying defect, each of which was reflected characteristically in the bone, teeth and blood, show that such deficiency may be present in the entire absence of caries. And the fact that the addition of corn oil to the basal diet, although it effected no abatement of its calcifying deficiency, produced a reduction of caries equivalent to that obtained with adequately calcifying diets, provides additional evidence pointing in the same direction. Finally, the circumstances that the degree of protective effect exerted by the calcifying foods and corn oil was not related to the amounts of these substances in the diet and particularly that a diet with fully adequate calcifying properties (56)

was not more effective in reducing caries than diets that produced somewhat imperfect calcification (66 to 69) suggest that these agencies operate by way of some mechanism other than that of calcification. We are hardly justified in concluding that these protective effects are exerted directly on the environment of the teeth rather than by way of systemic or metabolic channels, yet—excepting the influence of ultra-violet irradiation, which these results leave doubtful—the evidence does not invalidate the view that such may be the case.

SUMMARY AND CONCLUSIONS

This report deals with the results obtained with 286 animals, including 94 that received the (control) deficient caries-producing diet of rice passed through a 10-mesh sieve, dextrin and spinach and 192 that received various modifications of this diet intended to test the influence on the production of caries of calcifying agents and corn oil and the relative importance of the size of particles of rice and the two kinds of dietary calcifying defect. Of these animals 183 are included in the experimental and control groups described here in full, the remainder have been described elsewhere and were used here for comparative purposes. All the results are analyzed statistically. The results appear to warrant the following conclusions:

Caries is produced with the coarse rice basal diet either of the high calcium deficient type or of the low calcium type. The high calcium diet is not more effective in producing caries than the low calcium diet.

With both types of diet the incidence of caries becomes zero when the rice is ground to pass through a 100-mesh sieve.

Addition to the basal diet of cod liver oil or viosterol in corn oil as 2 per cent of the diet produced a definite reduction in caries, but did not prevent caries outright. The protective effect was not significantly augmented by increasing the dosage to 5 per cent of cod liver oil or viosterol in corn oil or by the further addition of calcium and phosphorus to a normal ratio, with levels normal or definitely high.

Irradiation of animals on the basal diet with ultraviolet rays in a dosage sufficient to produce improvement in calcification as great as that produced by 5 per cent of cod liver oil was less effective in reducing caries than feeding vitamin D, but this result is statistically inconclusive.

Addition to the basal diet of 2 per cent or 5 per cent of corn oil did not remedy the dietary deficiencies but nevertheless produced a significant reduction in caries statistically not distinguishable from that produced by feeding vitamin D in oil. Thus at least part of the protective effect of vitamin D-oil preparations appears to be contributed by the vitamin-free oil.

Within the groups that received diets with adequate or nearly adequate calcifying properties, individual variation in the incidence of caries was found to be unrelated to the blood levels of calcium or phosphorus. Likewise, the degree of protective effect against caries of the diets that were supplemented with calcifying agents was not related to the degree of their calcifying action. From the evidence as a whole it appears that the protective effect against caries exerted by the calcifying foods may be independent of the mechanism of calcification and possibly of metabolic influences generally.

SPONTANEOUS ARTERIOSCLEROSIS IN RATS

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In view of the complete lack of information in regard to the occurrence and frequency of spontaneous arteriosclerosis in rats (Duff¹), the following report may be of interest

In the course of routine histologic examinations of the organs of seventy-five rats used for experimental purposes, arteriosclerotic lesions were seen in the branches of the pulmonary artery in twelve. On account of the relatively small size of the lesions and the absence of serial sections it is probable that the incidence of arteriosclerosis of the pulmonary artery is higher than is apparent from these figures. In none of the other organs (brain, heart, ascending part of the aorta, liver, spleen, pancreas, adrenal gland, kidney, testis and bladder) were such changes observed. The rats were adult and all had been obtained from one dealer, but no information is available as to whether they belonged to one stock. As their death was the result of poisoning with a chemical that killed the animals within a few hours or days it is unlikely that an exogenous chemical factor was the cause of or contributed to the production of these lesions. They were found in the large and medium-sized branches of the pulmonary artery and were most frequently located at or near the spot of bifurcations of this vessel. While in the majority of the cases multiple plaques were shown in the walls of one or several vessels, occasionally only a single calcified focus was seen.

The greater number of the lesions consisted of cone-shaped calcified foci covered by endothelium and projecting into the lumen, being located in the subendothelial tissue and extending rather frequently into the media, which showed evidence of local hyaline degeneration. In some instances the calcified foci were indistinctly outlined streaklike formations involving only the media. Small hyalinized areas, sometimes containing minute central calcifications, were occasionally observed involving the media. The intima covering these areas was often thickened and the seat of cellular proliferation. It was noticed that there existed a more or less marked hypertrophy of the media in many of the smaller branches. Complete obliteration of the lumen of a medium-sized vessel with a large calcium deposit in the partially hyalinized and sclerotic wall was found in one instance. As these observations were made on paraffin sections exclusively no statements can be made as to the presence of deposition of lipoids in the vascular walls.

SUMMARY

Arteriosclerotic lesions in the walls of the branches of the pulmonary artery of the white rat are described

From the Haskell Laboratory of Industrial Toxicology

1 Duff, G Lyman Arch Path **20** 81, 1935

MOUSE LEUKEMIA

IX THE RÔLE OF HEREDITY IN SPONTANEOUS CASES

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The general question of the relative potency of heredity and environment must be replaced by a specific question for each specific case. For all degrees of relationship between these categories of controlling influences are now known to exist. Thus the distinction between so-called hereditary and nonhereditary traits is not clearcut nor does it represent a fundamental difference. If the hereditary variables are relatively strong, clearcut analyses in terms of specific genes are possible, when nonhereditary variables are strong, such analyses are rarely possible. Between these limits lie all degrees of genetic potency and hence all degrees of reliability of genetic interpretation.

In the case of leukemia the evidence from human records seems to indicate that heredity plays a very minor rôle (Ardashnikov,² Petri³). In mice, the leukemia of which shows the closest possible resemblance to the human disease and its variations, heredity can be shown to have an unquestionable influence.

The only published discussion on heredity in relation to the spontaneous leukemia of mice (Slye⁴) proposes a clearcut genetic interpretation on the basis of "perfect" and "nearly perfect" mendelian ratios. The results herein presented confirm the existence of a hereditary predisposition to leukemia, but also show that in this case nonchromosomal variables play such a conspicuous rôle that genetic ratios can be determined only by breeding tests of the individual mice in a segregating generation, not by their own somatic conditions, and, further, that even within the limits of the highly controlled experiments described

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1 Footnote deleted by the author.

2 Ardashnikov, S N. Proc M Gorky Med & Biol Research Inst **3** 169, 1934.

3 Petri, S. Acta path et microbiol Scandinav **10** 330, 1933.

4 Slye, M. Am J Cancer **15** 1361, 1931.

nonchromosomal variables cannot be eliminated as constant, for their effectiveness varies according to the genetic constitution of the mice

LEUKEMIC STRAIN C58

In earlier papers of the present series (Richter and MacDowell,^{5a} MacDowell and Richter⁶) reference has been made to the frequent occurrence of spontaneous leukemia in an inbred strain of mice designated as C58. This strain has provided most of the material for these studies. The ancestors of strain C58 were brought to the department of genetics in 1920 by Dr. C. C. Little. They were received from Mrs. Gray to whom were turned over the stocks of Mrs. Abby Lathrop. These stocks were derived from those studied in cooperation with Dr. Leo Loeb (Lathrop and Loeb⁷). The animals constituted a genetically heterogeneous group, they were of various colors—black, brown, yellow, brown agouti, subsequent inbreeding resulted in the isolation of several strains with characteristic traits, such as defects in development, a peculiar behavior pattern and the predisposition to leukemia. Since 1920 the matings in this strain have been between brother and sister, in the early generations blacks and browns occurred, but the direct ancestors of the recent generations have been homozygous black since the very earliest generations of inbreeding. Before the eighteenth generation several animals with greatly enlarged spleens were casually noted. Their occurrence in this strain was recognized as characteristic, but no particular attention was paid to them, and the records tell nothing about the incidence since most of the animals were killed as soon as breeding became reduced. This was usually early, for another characteristic of this strain was early maturity followed by unusually heavy deposition of fat and a short breeding period. A few animals with large spleens and livers were preserved, and from sections their condition was subsequently diagnosed by Dr. Alwin M. Pappenheimer as lymphatic leukemia.

In 1928, when the cooperative project on leukemia was undertaken by the two departments, all the animals of this strain not required for special experiments were permitted to die naturally until a group of 637 animals that lived at least six months was collected. Since the animals used in the special experiments were taken entirely at random, this was a purely random sample of the strain. These mice were born in generations 18 to 23. A second random sample consisted of 70 mice from generations 26 to 28, born in March and April 1931.

5 Richter, M. N., and MacDowell, E. C. (a) *Proc. Soc. Exper. Biol. & Med.* **26** 362, 1929, (b) *J. Exper. Med.* **53** 823, 1930.

6 MacDowell, E. C., and Richter, M. N. *Biol. Zentralbl.* **52** 266, 1932.

7 Lathrop, A. E. C., and Loeb, L. *J. Exper. Med.* **28** 475, 1918.

DIAGNOSES

All these mice died and their disorders were diagnosed by one of us (M N R) with the aid of the gross autopsy records and microscopic sections of the principal organs and tissues fixed in Zenker's fluid and stained with hematoxylin and eosin

The occurrence in this highly homogeneous strain of mice of various conditions involving malignant leukocytic infiltrations, recognized by different descriptive names, justifies the use of one term to cover the group. In the absence of any term entirely satisfactory for this purpose we have used the term "leukemia" as a matter of convenience, believing that definitive terminology can be established only at the completion of experimental analysis. Thus in the tables and pedigrees leukemia and related conditions are all classed as leukemia.

Serious difficulty was encountered in the diagnosis of certain conditions owing to rapid changes post mortem, and to death from other causes with leukemia in some incipient stage. Occasionally other puzzling histologic conditions were encountered that defied diagnosis. Thus a class of doubtful cases arose. In the first sample of 637 mice 99 cases (15.5 per cent) were doubtful, in 63 per cent of these the doubt was manifestly due to changes post mortem. Thirty-five of the doubtful cases were considered to be probably cases of leukemia, and 20, probably cases of nonleukemic disorders, no probability was indicated for the remaining 44 cases. In order to minimize the doubtful cases the animals in the second sample were killed as soon as clear clinical indications of leukemia were found or on approach of a moribund condition. In this way definite diagnoses were obtained for all except 2 mice which died unexpectedly. The virtual elimination of doubtful cases in this sample may indicate that slight changes post mortem can lead to doubt or that doubt may be due to terminal complications of various sorts. Breeding tests (table 4) showed that mice with doubtful diagnoses were not genetically different from those with positive diagnoses. Thus doubtful cases as a whole appear to represent an accidental class without significance for this study. So far as this is true they form a random sample, and their elimination from the calculations of the incidence of leukemia appears to be the least unsatisfactory method of dealing with them. When so calculated the incidence of leukemia in the two samples from strain C58 becomes 90.1 per cent and 85.3 per cent of the definite diagnoses.

In the great preponderance of the cases the leukemia was of the lymphatic type. Out of a grand total of 543 cases in which leukemia was positively diagnosed, in 450 the condition was lymphatic (in 19 of these, lymphosarcomatous), in 6, myeloid, and in 87, doubtful as to cell type. In 55 of the doubtful cases the condition was called "prob-

differences This can be checked in two ways If there are no genetic differences between leukemic and nonleukemic mice in this strain the nonleukemic mice should be scattered at random through the different families and branches of the pedigree and the offspring of the nonleukemic parents should give the same incidence of leukemia as the offspring of the leukemic parents

TABLE 2A—*The Leukemic, Nonleukemic and Doubtful Offspring in the First Sample of Six Hundred and Thirty-Seven Mice from Strain C58, Generations 18 to 22, Classified by Fathers*

Generation	Fathers*	Offspring†		
		+	—	?
16	38208			
17	40086			
18	A 43833	8	0	0
19	48387	3	1	0
19	46864	19	3	2
20	50418	43	7	9
21	52437	17	1	2
22	53449	10	2	3
23	55252	12	2	0
22	53947	8	1	1
21	52649	28	2	10
22	53641	8	0	2
22	54296	7	0	1
22	53567	3	1	0
21	53376	5	1	0
22	54398	2	1	5
21	52708	29	5	5
22	54101	5	0	1
22	53705	5	0	1
20	52489	28	1	2
21	53320	8	1	1
		248	29	45
18	B 43672	6	1	2
19	46617	25	5	8
20	49372	28	1	9
21	52628	3	0	1
21	53245	12	1	2
20	52447	2	0	0
20	49159	26	1	3
21	52535	11	0	3
21	52769	9	2	1
20	53503	0	1	1
19	49717	34	2	6
20	52496	40	4	5
21	54063	2	0	1
		198	18	42
17	42101			
18	O 45855	4	2	0
19	49917	34	3	10
20	53250	1	1	2
		39	6	12
Total		485	53	99

* A son is below his father and one step to the right

† The plus sign means leukemic, the minus sign, nonleukemic, the question mark, doubtful as to leukemia

Evidence on these points is given in tables 2 to 4 Table 2 summarizes the data from the first group of 637 mice according to individual fathers and table 3 according to generations It is clear that the nonleukemic as well as the doubtful leukemic offspring were distributed through all the pedigree, this is direct evidence of genetic homogeneity

Table 4 classifies all the matings according to the diagnoses of conditions in the parents. While the number of nonleukemic parents is necessarily small, it is none the less certain that matings involving 1 or 2 nonleukemic parents yielded fully as large proportions of leukemic offspring as matings between 2 leukemic parents. Thus by direct breeding test nonleukemic animals in strain C58 were shown to be genetically the same as the mice that died of leukemia. From this it follows that heredity was strong enough to lead to leukemia in most of the mice of strain C58, but 10 per cent of them met conditions that prevented the manifestation

TABLE 2B—*Leukemic, Nonleukemic and Doubtful Offspring in the Second Sample of Seventy Mice from Strain C58, Generations 26 to 28, Classified by Fathers, Giving the Connection with the Earlier Sample*

Generation	Fathers	Offspring		
		+	—	?
21	52649			
22	54296			
23	56619			
24	61398			
25	66388			
26	70130			
27	77071	4	0	0
25	64450			
26	67260			
27	70324	4	1	0
21	52708			
22	56301			
23	60364			
24	61703			
25	64324			
26	68890			
27	72085	7	1	1
28	78568	4	0	1
26	72948	12	4	0
27	69495			
28	77942	13	0	0
25	64378			
26	67143			
27	72884	5	0	0
26	68767			
27	77407	5	4	0
27	77412	4	0	0
Totals				
Generation 26		12	4	0
Generation 27		29	6	1
Generation 28		17	0	1

of the hereditary tendency. One could say that in strain C58 heredity was 9 times as potent as nongenetic variables in controlling the incidence of leukemia. It might be supposed that death from some independent cause before leukemia could manifest itself accounts for the negative cases. This cannot have played any primary part since the animals not showing leukemia tended to live longer than those with the disease (average age at death of 63 nonleukemic animals, 495.5 days, average age of 543 leukemic mice, 396.4 days), 55 leukemic mice died earlier than the youngest nonleukemic one, and the oldest 3 mice were without sign of this condition, 47 per cent of the leukemic mice died

before 360 days, as compared with only 22 per cent of the mice free from this disease

All the mice of strain C58 inherited a tendency toward malignant change in the leukocytes. But even among those with leukemia there was indication of the action of nongenetic influences since different mice showed considerable range in the malignant manifestation. Transmission experiments have proved the existence of lines of malignant lymphocytes with many different traits (Richter and MacDowell,^{5b} Victor and

TABLE 3—*Spontaneous Leukemia in Strain C58. A Summary of the Diagnoses According to Branch of Strain and According to Inbred Generation*

	Ancestor	Diagnoses			Percentage of Definite Diagnoses Positive for Leukemia
		+	—	?	
♂ 43833		248	29	45	89.5
♂ 43672		198	18	42	91.7
♂ 45855		39	6	12	86.7
Generation					
	18-19	133	17	28	88.7
	20	168	16	31	91.3
	21	124	13	26	90.5
	22-23	60	7	14	89.6
	18-23	485	53	99	90.1
	26-28	58	10	2	85.3
Totals					
	Males	240	30	54	88.9
	Females	303	33	47	90.2
		543	63	101	89.6

TABLE 4—*A Classification of the Offspring in Strain C58 According to the Presence or Absence of Leukemia in the Parents*

Parents	Offspring			Percentage of Definite Diagnoses Positive for Leukemia
	+	—	?	
+ × +	286	36	59	88.8
+ × ?	53	5	13	91.4
? × ?	11	0	1	100.0
+ × —	55	5	12	91.7
— × ?	4	0	0	100.0
— × —	10	0	3	100.0

Potter,⁸ Furth, Seibold and Rathbone⁹), indications are strong that leukemic cells from different spontaneous cases are inherently different. Given uniformity of the strain, the source of these differences is apparently nongenetic.

Since the relation of leukemia to other neoplasms has been a matter of discussion, table 5 is presented, showing the distribution in the large sample from strain C58 of other neoplasms according to the diagnosis in regard to leukemia and according to the father. The neoplasms were

⁸ Victor, J., and Potter, J. S. Proc Soc Exper Biol & Med **30** 523, 1933

⁹ Furth, J., Seibold, H. R., and Rathbone, R. R. Am J Cancer **14** 3, 1933

distributed generally through the pedigree without respect to the occurrence of leukemia. Calling the offspring of one father by his sibs a family, one finds that of 35 families 17 disclosed no neoplasm besides leukemia while 18 each included 1 or 2 mice with the certain or questionable presence of some other neoplasm. Special attention should be called to 2 bone tumors as well as to the scarcity (2 cases) of mammary car-

TABLE 5—Occurrence of Other Neoplasms in Leukemic, Nonleukemic and Doubtful Offspring Classified According to Parentage

Gene ration	Fathers	Neoplasms in Leukemic Offspring	Neoplasms in Nonleukemic Offspring	Neoplasms in Offspring in Which Leukemia Was Doubtful
16	38208			
17	40086			
18	43883			
19	48387			
19	46864	Carcinoma of lung		Adenoma of liver
20	50418			
21	52437			Sarcoma
22	53449			
23	55252		Sarcoma (?) of mesenteric node	
22	53947		Neoplasm (?) of spleen	
21	52649	Carcinoma of breast		
22	53641			
22	54296			
22	53567	Carcinoma of lung		
21	53376			
22	54398			
21	52708			
22	54101			
22	53705			
20	52489	Carcinoma of lung	Carcinoma of kidney	
21	53320	Hemangioma and adenoma of liver		
18	43672	Adenoma of liver		
19	46617	Carcinoma of lung		Adenoma of liver
20	49352	Sarcoma (?) of liver		Epithelioma
21	52628			
21	53245			
20	52447	Carcinoma of breast		
20	49159	Adenoma of liver (?)	Adenoma of bile ducts	
21	52535			Chondro osteosarcoma
21	52769		Cylindroma of parotid gland	
20	53508		Sarcoma (?) of node	
19	49717			
20	52496	Hemangioma of liver		
		Osteoblastic sarcoma		
21	54063			
17	42101			
18	45855			
19	49917	Hemangioma of pancreas		
20	53250			
		13 (485) = 2.7%	6 (53) = 11.3%	5 (99) = 5.1%
		Total 24 (637) = 3.8%		

cinoma. The appearance of such sporadic cases of various neoplasms is commonly classified as nongenetic whereas it would be more correct to say that the constitution of mice of strain C58 is such that only under very unusual circumstances will such and such a neoplasm other than leukemia develop.

STRAIN STORRS-LITTLE

In order to prove the existence of hereditary influence it is necessary not only to demonstrate the occurrence of a trait in successive

generations of a given strain but to show that under the same external conditions strains with different constitutions give different results. Whereas several strains bred side by side with C58 have never shown a single case of leukemia, only one, StoL₁ (Storris-Little), has been extensively studied. This is the strain that was not susceptible to inoculation with leukemic cells of line I (MacDowell and Richter⁶). The records of 306 autopsies between generations 14 and 29 of brother and sister mating have been studied. The outstanding neoplastic characteristic of this strain of pink-eyed dilute-brown mice was the incidence of mammary carcinoma. Among 87 autopsies on females in generations 14 to 19 were found 44 cases of mammary carcinoma, that is, 50.6 per cent of all females showed this neoplasm in comparison with 0.5 per cent of the females from strain C58. However, after generation 19 the incidence of mammary carcinoma was suddenly and inexplicably reduced to 4.7 per cent (in 169 females). There was no change in care, diet or room and there was no elimination of branches of the pedigree that could be connected with this change. For the whole series (256 females) the incidence of mammary carcinoma becomes 20.3 per cent. A few other tumors were scattered through the pedigree, most of these were carcinoma, but four (1.3 per cent of all autopsies) were diagnosed as lymphatic leukemia.

The isolated cases of leukemia in StoL₁ correspond in frequency to the isolated cases of mammary carcinoma in C58, they were non-genetic in the sense that they appeared only under very unusual conditions. But should other strains of mice be found the constitutions of which do not permit the appearance of either of these types of neoplasm under any conditions, they would provide a basis for measurement of the genetic influence in such so-called nongenetic cases.

Thus with a common environment there is established a difference between these two strains in the incidence of leukemia which is to be accredited to heredity and which is represented roughly by 90 per cent and 1 per cent. With these facts in hand a genetic analysis of this difference can be made by study of the segregation in generations following a cross between these two strains.

FIRST GENERATION HYBRIDS BETWEEN C58 AND STO L₁

If a strain is genetically homogeneous all germ cells are genetically alike so that in the first generation of a cross between two homogeneous strains every fertilized egg must be genetically like every other one. Thus in the cross of strain C58 and strain StoL₁ all hybrids in the first generation are as genetically uniform as the parent strains. Table 6 shows that of 106 offspring with definite diagnoses from C58 fathers and StoL₁ mothers 42.5 per cent had leukemia, a reduction of virtually one half in the incidence of leukemia. This intermediacy in the incidence

of leukemia is not reflected in the character of the leukemia, for the cases cannot be distinguished from those in the pure bred strain, nor does this constitute an example of intermediate or blending inheritance, for in blending inheritance differences in genetic condition are indicated directly in somatic differences. Since the mice in this generation are genetically alike the presence or absence of leukemia must be decided by nonchromosomal variables. Thus in the first hybrid generation, as in the pure bred strain C58, the somatic manifestation of the individual mouse is not a direct measure of its genetic constitution. But in this generation the relative influence of nongenetic variables is more than 5 times as strong as in the inbred mice of strain C58. Closely parallel results from hybridization of strains of mice characterized by high and low cancer rates have been reported by Lathrop and Loeb.⁷

TABLE 6—*First Generation (F₁) from Reciprocal Matings Between Eleven Males and Seventeen Females from Strain C58 and Six Males and Twenty-One Females of Strain StoL₁*

Sex	Number of Offspring from						Percentage of Definite Diagnoses Positive for Leukemia in Mice from		
	C 58 Mothers			StoL ₁ Mothers			C 58 Mothers	StoL ₁ Mothers	Difference
	+	—	?	+	—	?			
Male	41	32	13	26	38	13	56.2	40.6	15.6
Female	45	21	14	19	23	21	68.2	45.2	23.0
Total							61.9	42.5	$19.4 \pm 4.3 = 4.5 \times P.E.$

Among the nonchromosomal variables which modify the manifestation of leukemic heredity one important influence is transmitted through the mother alone. The results so far considered have not been subject to this influence since the discussion has been based on hybrids receiving the leukemic inheritance from their fathers. The corresponding figures for transmission through the mothers are 19.4 per cent higher, a difference of unquestionable statistical significance. A question of sex linkage naturally arises, for in a cross involving sex linkage reciprocal matings give different results in the first generation, but these differences are confined to the males alone. In this case the female offspring show as much or even more difference than the males. Thus sex linkage is eliminated.

Length of life (table 7) gives further evidence of difference between offspring from the reciprocal matings of this cross. The average difference, 138 days, is twelve times its probable error. Sex linkage is not involved since females show as great a difference as males (difference for males, 132.3 days, for females, 144.6 days).

Again evidence of nonchromosomal maternal transmission appears in the case of transmission of mammary carcinoma from strain StoLi. Excluding matings with StoLi after the nineteenth generation, when the incidence of mammary carcinoma became reduced, the difference (39.7 per cent more mammary carcinoma in daughters from StoLi mothers than in those from C58 mothers) is statistically significant (7.7 times the probable error). Thus in one form of the cross is found an excess of leukemia, in the other, an excess of carcinoma. This negative correlation between the two types of neoplastic disease indicates specific difference but similarity in the type of transmission. Some indication of such maternal influence on the cancer rate in hybrids was given by Lathrop and Loeb.⁷ More recently several crosses reported by the Roscoe B. Jackson Memorial Laboratory (through C. C. Little¹⁰) involving wide differences in the incidence of mammary tumors show striking differences in the first generation from reciprocal matings.

TABLE 7—*Average Length of Life in Days*

Parents	Offspring				
	Leukemic	Nonleukemic	Males*	Females*	Total
C 58	396.4 (543)†	495.4 (63)	447.5 (327)	384.1 (380)	413.4
StoLi		563.9 (302)	548.2 (50)	586.5 (252)	563.9
StoLi/C 58	729.1	659.6	684.4	682.4	683.5
C 58/StoLi	554.3	503.7	552.1	537.8	545.2
Difference	174.8 ± 17.6	155.9 ± 20.0	132.3 ± 15.5	144.6 ± 17.1	138.3 ± 11.5
Difference/P.E.	9.9	7.8	8.5	8.4	12.0

* Includes positive, negative and doubtful cases

† The number of mice is given in parentheses

The mechanism of this maternal transmission has not been demonstrated, but the probability is strong that the cytoplasm of the egg is involved since this constitutes the outstanding difference between the contribution of male and female germ cells. There remains, however, the possibility that some influence is transmitted through the placenta during gestation. The influence of mother's milk has been largely eliminated by fostering experiments in which C58 mice were nursed by foster mothers in nonleukemic strains and StoLi mice by C58 mothers without reducing the incidence of leukemia in C58 mice or inducing it in StoLi mice.

Whereas the expression of both leukemia and mammary carcinoma is subject to nonchromosomal influence transmitted by the mother, when this is eliminated there remain in each case other unidentified sources of nonchromosomal and entirely nongenetic influence the effectiveness of which is even greater.

Although clearcut dominance and recessiveness have been called into the discussion of the manner of inheritance of leukemia by Slye,⁴ it is certain that no such phenomenon is represented by these results. One might indeed consider that leukemia is a recessive trait stimulated into expression in hybrids by nongenetic variables, but one could equally well consider that it is a dominant trait the expression of which is inhibited by certain nonchromosomal variables. Thus the terms "dominance" and "recessiveness" have no meaning in this case. This conclusion agrees with an enormous mass of genetic evidence indicating that the concept of complete dominance has little if any general significance (Hogben¹¹).

BACK CROSS TO STO LI

Just as breeding tests gave direct verification of the evidence that pure bred mice of strain C58 were genetically alike whether they had leukemia or not, the genetic uniformity of mice in the first hybrid gener-

TABLE 8—*Breeding Tests of Hybrids (F₁) with Different Diagnosis**

Diagnosis of Hybrid Parents	Offspring			Percentage of Definite Diagnoses Positive for Leukemia
+	37	37	10	50.0
—	16	21	7	43.2
?	21	27	12	43.8

* Pregnancy of F₁ females from C58 mothers and StoLi fathers crossed back to StoLi males. The F₁ males used in the back cross were all leukemic.

ation has been directly substantiated by comparing the offspring of leukemic and nonleukemic hybrids crossed back to the pure bred strain StoLi. Table 8 gives the figures for this comparison.

Table 9 gives further evidence of nonchromosomal maternal influence. For in this back cross generation the incidence of leukemia is significantly higher (26.7 per cent) when the hybrid parent is the mother.

In back crosses genetic segregation occurs. Although the individual mice in the first hybrid generation are alike, the germ cells they produce are diverse, including all possible combinations of the genes by which the parent strains differ. So far as genetic constitution is directly expressed in the somatic conditions of the individual mice the back cross provides evidence of the number of genes by which the two pure strains differ. Thus the mice in this generation were divisible according to color of hair into eight equal groups corresponding to the eight possible combinations of the three pairs of genes influencing the color of hair by which C58 and StoLi differ. In the same way have occurred the segregation and recombination of all other genetic determiners,

11. Hogben, L. *Nature and Nurture*, New York, W. W. Norton & Company, Inc., 1934.

whether for structural, physiologic or pathologic traits. In regard to leukemia it is obvious that the genetic constitution is not directly expressed somatically, a leukemic and a nonleukemic mouse may have the same constitution, while two leukemic mice may be genetically different.

Thus the incidence of leukemia in the back cross does not give a basis for determining the number of units responsible for the hereditary difference between C58 and StoL₁. Aid in such a genetic analysis would be afforded by findings that some gene influencing the occurrence of leukemia was located in the same chromosome as a gene for color of hair. This linkage would be indicated by irregularity in the distribution of leukemic mice among the eight color classes. However, the classification of data in this way showed essential uniformity in the distribution of leukemia among the color classes.

TABLE 9—Incidence of Leukemia in Back Cross Mice Classified According to the Sex of the Hybrid Parent (Matings of StoL₁ with Hybrids from C58 Mothers and StoL₁ Fathers)

Hybrid Parents	Offspring			Percentage of Definite Diagnoses Positive for Leukemia	Difference P E
	+	—	?		
Father	19	77	18	19.8	
Mother	74	85	29	46.5	
Difference				26.7 ± 3.8	7.0

Animals from the reciprocal matings of the back cross also differ significantly in longevity. The average length of life (559 days, 188 mice vs. 652.4 days, 114 mice) is 93.4 ± 13.9 days longer (67 times the probable error) when the mother is pure-bred StoL₁. Thus in two hybrid generations longer life is associated with less leukemia, this is also the case in the two pure-bred strains. But this relationship is not directly causal because (1) the difference in length of life is shown by the leukemic as well as by the nonleukemic mice, (2) from pure StoL₁ fathers the first hybrid generation and back cross animals show almost exactly the same length of life as the pure StoL₁ animals, 545, 559 and 564 days, respectively, yet they give 61.9, 46.5 and 1.3 per cent leukemic mice, (3) first generation hybrids with StoL₁ mothers and back cross animals with StoL₁ fathers give nearly the same proportion of leukemic mice, but the average length of life differs by 124 days. In other words, leukemia transmitted through fathers gives midparental incidence in both hybrid generations, whereas greater longevity transmitted through fathers gives the same length of life in the two hybrid generations as in the pure-bred longer-living strain. Transmitted by mothers the longevity in the two hybrid generations is significantly greater than in the pure-bred longer-living strain.

The simple fact is that with the maternal factor barred the incidence of leukemia is reduced about half when the total heredity from strain C58 is reduced one half, when this is again reduced by one half by the back cross the incidence of leukemia is again reduced about half. The rôle of heredity is unquestionable and can be expressed in quantitative terms. This is the type of correlation between heredity and somatic traits observed by Galton, on the basis of such evidence a general statistical description of heredity called Galton's law was formulated. However, this takes no account of the transmission of traits in terms of genes. With results of this type many theoretical interpretations in terms of genes could be proposed, but the only possibility of obtaining evidence to distinguish between these interpretations lies in testing the genetic constitution of every individual mouse in the back cross by its transmission of leukemia to its offspring in a second back cross. Classified on such a basis the animals in the first back cross would reveal a genetic ratio capable of significant interpretation. Unfortunately the program for these experiments did not include such a second back cross, which will have to await a repetition of the original crossing.

COMMENT

Strains of mice characterized by spontaneous conditions similar to the leukemia in strain C58 have been reported, respectively, by Dobrovolskaia-Zavadskaia,¹² McCoy-Hill¹³ and Slye.⁴ The first mentioned strain, in comparison with various other strains in the same laboratory which together gave an incidence of 6 per cent, showed an incidence of 20 per cent (total number of mice, 143). The second strain had been inbred since 1920 under the direction of Dr. T. Brailsford Robertson, in a sample of 216 mice 134 (62 per cent) showed some form of lymphoid or myeloid hyperplasia. Of these, 50 per cent had lymphoid, 14 per cent myeloid, 23 per cent atypical or mixed types of hyperplasia, and the remainder had what was called Hodgkin's disease. Slye gave a pedigree mostly of brother and sister matings showing the causes of death in 152 mice, of which 36 (23.7 per cent) had some form of leukemic disease, however, in one branch the incidence was much higher. Four successive generations of this branch consisted of (1) 3 leukemic and 3 nonleukemic mice, (2) 5 leukemic and no nonleukemic mice, (3) 3 leukemic and no nonleukemic mice, and (4) 3 leukemic and no nonleukemic ones. Slye is the only author who deals directly with the genetics of mouse leukemia, she believes that all neoplastic conditions, including leukemia,

¹² Dobrovolskaia-Zavadskaia, N. *Compt. rend. Soc. de biol.* **109** 339, 1932.

¹³ McCoy-Hill, F. *J. Cancer Research* **14** 325, 1930.

depend on a single recessive mendelian gene. However, geneticists do not concur in this opinion since the evidence necessary for such a conclusion has not been given.

Her case rests on the accumulation of instances. The paper on leukemia is based on 36 cases of leukemic diseases in one pedigree, 14 others are mentioned specifically, while 924 cases in other pedigrees in the first 50,000 autopsies are not accounted for in any way. No explanation is given of this abstemious use of available data, or of why this particular set was presented. Such selected evidence might justify a working hypothesis, but an acceptable conclusion requires the elimination of alternative hypotheses by experimental tests.

Although dealing with an experimental animal and using the terminology of genetics, Slye has not used the basic genetic test. Cross breeding is the critical tool for analysis of genetic differences. It consists of deliberate matings between animals from strains showing consistent differences, the generations designated as F_1 and F_2 , etc., are defined by the experimental procedure, the results can be checked by repetitions with different animals. In contrast to this the procedure of Slye has been to breed the mice and after all were dead to decide from the results which mating was a "cross." The data presented concern an inbred pedigree of 11 generations originating in a single pair of mice with a "common house mouse" as father in one of the matings in the second generation (a mating that was not referred to as a "cross"). All other matings shown on the charts were between brother and sister. The failure to submit her hypothesis to the tests of a cross was not due to the lack of a contrasted strain, for she states "There have been very many strains in the laboratory completely free from any form of these diseases. Into these strains I have never bred any leukemia nor has it arisen sporadically in such strains" (conclusion 2, p. 1384).

But before the number of genes can be determined by the segregation following a cross it is necessary to delimit the influence of nongenetic variables. Slye's inbred pedigree represents the progress of genetic purification which must precede an evaluation of nongenetic variables, but her data stop at the point at which such an evaluation might have begun. With the relative potency of genetic and nongenetic factors unestablished, any interpretation of her data must remain purely hypothetical. If nongenetic factors influence the results, as Slye has admitted in certain places and as the present study amply affirms, a single gene will not yield conventional ratios, and so the close similarity of certain of her results to mendelian ratios serves to deny the very hypothesis which she claims they verify.

Slye offers no interpretation of the genetic differences between strains in the frequency and in the type of neoplasm, although she is apparently dealing with such differences. She stresses the point that leukemia occurs in strains along with other tumors, but also states that she has tumor strains in which leukemia has never appeared. In spite of this indication that the genetic control of leukemia differs from that of other neoplasms, the genetic conclusions on leukemia are given only in terms of malignancy and nonmalignancy.

CONCLUSIONS

The predisposition to leukemia and related diseases in mice of strain C58 is specifically heritable.

In crosses with strain StoL₁ the incidence of leukemia is roughly correlated with the proportion of total heredity from strain C58.

In the incidence of leukemia nonchromosomal variables play a rôle that grows increasingly important as the proportion of total leukemic heredity is reduced.

Among nonchromosomal influences that act as deciding factors in the incidence of leukemia an important one is transmitted by the mother.

Under these conditions somatic expression of leukemia in a segregating generation cannot disclose genetic ratios. Such ratios can be obtained only by classification according to progeny tests.

THE NATURE OF THE ANEMIA IN ACUTE LEUKEMIA

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One of the most characteristic symptoms of acute leukemia is the severe and rapidly progressing anemia, which in the subleukemic and aleukemic form often causes considerable difficulties in differential diagnosis (Kracke,¹ Naegeli,² Parker Weber,³ Rosenthal⁴ and others) This anemia may develop during the course of the disease, or it may precede it for weeks or even months In some cases the disease first presents itself under the clinical and hematologic picture of aplastic anemia or hemorrhagic aleukia Either spontaneously or under the influence of antianemic treatment the anemic condition may improve temporarily, and the blood count may show practically normal values The second attack usually is typically leukemic, the treatment that prompted the first remission is of no avail, and the disease terminates rapidly in death (Colarizi,⁵ Klein,⁶ Knudsen,⁷ Scholtz,⁸ Ullrich⁹ and others) With the diagnosis of leukemia established, reexamination of the slides of the blood made during the first attack may reveal single very immature white blood cells, which at the first examination were mistaken for lymphocytes These cases of severe initial anemia and leukopenia have induced some investigators to assume that there are pathogenic relations between the aleukias and leukemia, considering the two as different reactions to the same etiologic agent (Ederle and Esche,¹⁰ Segerdahl,¹¹ Rittmann,¹² Strumia¹³ and Ullrich⁹)

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1 Kracke, R R, and Garver, H J A M A **104** 697, 1935

2 Naegeli, O Blutkrankheiten und Blutdiagnostik, ed 5, Berlin, Julius Springer, 1931

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At the present time the severe anemia of acute leukemia is generally attributed to the replacement of erythropoietic tissue by leukemic tissue. Whipple and Robscheit-Robbins,¹⁴ who analyzed the liver chemically in a series of cases of acute leukemia, did not find an increase in the iron content and concluded that there was no or very little evidence of excessive destruction of the blood. On the other hand, von Kress¹⁵ pointed to the hemosiderosis of the blood-forming organs and to the increased excretion of urobilin in the feces and in the urine as indicating an excessive destruction of erythrocytes. The hemorrhages, which are common in cases of acute leukemia, may contribute to the anemia, but there is no constant parallelism between the progression of the anemia and the intensity of the hemorrhagic diathesis. Reference has also been made to the possibility that hemotoxic substances may be produced by the leukemic tissue (Hirschfeld¹⁶). In an earlier paper¹⁷ I described a marked hyperplasia of the erythropoietic tissue of the bone marrow and extramedullary foci of erythropoiesis in many cases of leukemia. I have also stressed the frequent occurrence of considerable hemosiderosis, suggesting that excessive destruction of the red blood cells rather than lack of erythropoietic tissue may account for the anemia. This conception is supported by the fact that in some cases of acute leukemia the patient dies of severe anemia before the leukemia has developed fully. Even though the blood picture may be typical of leukemia, microscopic examination of the organs shows only slight leukemic changes and a considerable amount of normal myelopoietic tissue. During the last three years I have observed five patients whose cases fit into this description, and since these cases may cast light on the obscure pathogenesis of acute leukemia, they are reported here in detail.

REPORT OF CASES

CASE 1—History and Course—A Negro boy aged 4 years was first admitted to a surgical ward because fracture of the skull was suspected. Three days before admission the child fell against a table and remained unconscious for ten minutes. Three days later the nose started to bleed, and vomiting followed. The next day the stools were black. The child was restless during the night and the next morning appeared drowsy.

On his admission the temperature was 98.8 F, the respiratory rate 28 and the pulse rate 170. The liver could be palpated 2 fingerbreadths below the costal arch, and the spleen extended for 2 fingerbreadths below the costal arch. In the

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skin there were numerous hemorrhagic areas ranging from the size of a pin-point to a diameter of 2 inches (5 cm). The axillary and inguinal lymph nodes were palpable.

The Wassermann and Kahn reactions were negative. The blood showed 868 mg of uric acid per hundred cubic centimeters. The urine was normal. Blood was noted in the stools.

Repeated intramuscular injections of whole blood had no effect, and the child died on the third day after admission, after an illness of apparently only six days.

Examination of the Blood—The blood count showed erythrocytes, 1,260,000, hemoglobin, 42 Gm (Newcomer), reticulocytes, 48 per cent, leukocytes, 33,800, hemocytoblasts (stem cells), 272 per cent, large lymphocytes, 272 per cent, small lymphocytes, 192 per cent, promyelocytes, 08 per cent, metamyelocytes, 24 per cent, neutrophilic leukocytes, 8 per cent, plasma cells, 08 per cent, and monocytes, 144 per cent. There were marked poikilocytosis and anisocytosis, many polychromatophils and 7 normoblasts and 1 erythronorm, per hundred leukocytes. There were 82,000 platelets. The bleeding time was six and one-half minutes.

Gross Postmortem Examination—There were evidences of severe anemia. Petechial hemorrhages were observed in the skin, lungs, pleura, epicardium, renal pelvis, brain and leptomeninges. There was fatty degeneration of the myocardium, liver and kidneys. The spleen was hyperplastic, weighing 110 Gm and measuring 11 by 8 by 4 cm. The thymus weighed 12 Gm. Slight hyperplasia and deep purple-red discoloration of the peripheral, thoracic and abdominal lymph nodes were noted. The largest lymph node was 10 mm in diameter.

Histologic Postmortem Examination—The bone marrow (femur, sternum) was extremely congested with blood. There were only a few fat cells and the majority of the sinusoids and capillaries were collapsed, the blood cells being located outside the preformed blood spaces. Enclosed in the dense accumulations of erythrocytes were small islands of nucleated elements, and an occasional dilated sinusoid was filled with those cells. The nucleated elements occupied approximately 17 per cent of the marrow space. A differential count showed undifferentiated lymphoid cells with a finely reticulated chromatin structure of the nucleus and a distinct rim of homogeneous basophilic cytoplasm (stem cells or hemocytoblasts), 114 per cent, myeloblasts, 08 per cent, neutrophilic myelocytes (the majority with poorly defined and disintegrating granulation), 8 per cent, neutrophilic leukocytes, 3 per cent, oxyphilic myelocytes, 8 per cent, oxyphilic leukocytes, 32 per cent, erythronorm, 28 per cent, erythroblasts, 16 per cent, normoblasts (many very large ones with polychromatic or orthochromatic cytoplasm), 462 per cent, megakaryoblasts, 04 per cent, megakaryocytes (poorly preserved, with pyknotic nuclei and homogeneous cytoplasm), 1 per cent, lymphocytes, 1 per cent, plasma cells, 02 per cent, monocytoid cells, 2 per cent, free histiocytes with engulfed red blood corpuscles, 3 per cent.

The liver, lymph nodes and especially the spleen revealed marked myeloid metaplasia. In the spleen, which was greatly congested, erythropoiesis predominated. The liver showed erythropoiesis and granulopoiesis in and about the portal sinusoids and in the periportal septums. The lymph nodes contained neutrophilic and oxyphilic myelocytes and megakaryocytes. In addition to the myeloid cells which showed the tendency to mature and did not differ from the cells seen in cases of reactive extramedullary myelopoiesis, there were groups and nests of undifferentiated cells which were identical with those described in the

bone marrow They gave a negative reaction for oxidase and could be readily differentiated from lymphocytic elements as well as from myeloblasts, which give a positive reaction for oxidase They were more numerous in the liver than in the spleen and were scanty in the medullary cords of the lymph nodes The purple-red discoloration of the lymph nodes was due to the filling of the sinusoids with red blood corpuscles, many of which had been taken up by the swollen and proliferated reticular endothelium of the sinusoids There was also a moderate erythrophagocytosis by the Kupffer cells of the liver In the medulla of the lymph nodes the small lymphocytes showed a peculiar crenation and segmentation of the nuclei, which was also found in the small round cells of the thymus The lymphatic tissue of the pharynx and intestinal tract was normal The kidneys, lungs and glands of internal secretion were normal In the brain the hemorrhages were located about small blood vessels Between the walls of the vessels and the hemorrhages there was often a zone of lymphoid round cells or a zone of necrosis with swollen microglia cells and occasional lymphoid cells

The reaction for iron was as follows Kupffer cells, +++, hepatic cells, +, splenic pulp, ++, lymph nodes, +++, bone marrow, +, and kidney, 0

CASE 2—History and Course—A white woman, a housewife aged 38, for the past seven weeks had been suffering from frequent vomiting The vomitus consisted of bright red blood or of coffee-ground material The medicine prescribed by a physician had no effect Occasionally there was also bleeding from the gums A few days before admission the patient started to cough and became severely constipated Epigastric distress developed, but there was no selective dyspepsia or any relation to food Since childhood the patient had bruised easily She had had three normal deliveries Pneumonia developed several years ago Pelvic laparotomy was performed seventeen years prior to admission

On admission the temperature was 100 F, the pulse rate 112 and the respiratory rate 28 The blood pressure was 130 systolic and 90 diastolic The patient was pale, and the tongue and gums were covered with a film of loosely coagulated blood There were retinal hemorrhages in the right eye The teeth were in poor condition The tonsils were moderately enlarged Over the apex of the heart a soft systolic murmur was heard The lower pole of the spleen could be felt below the costal arch Over the extremities there were scattered petechiae, and around wounds from needle punctures large ecchymoses had developed

The urine was normal The Wassermann and Kahn reactions were negative Roentgen examination of the gastro-intestinal tract revealed no abnormalities

During the patient's stay in the hospital the temperature rose at times to 105 F The hematemesis persisted, and she became rapidly weaker She expired five days after admission to the hospital after an illness of about seven weeks Two blood cultures were sterile

Examination of the Blood—The blood count showed, erythrocytes, 1,800,000, hemoglobin (Sahl), 22 per cent, leukocytes, 10,600, neutrophilic myelocytes, 3 per cent, juvenile neutrophilic leukocytes, 4 per cent, band forms, 10 per cent, segmented forms, 37 per cent lymphocytes, 30 per cent, monocytes, 15 per cent, and basophils 1 per cent The neutrophilic granulocytes were distinctly smaller than normal Some of them were greatly shrunken The granulation was abnormal Many cells had a deeply stained granulation, while in some of the cells the granules were transformed into rose-red droplets The monocytes had a heavy azure granulation Marked anisocytosis and poikilocytosis and single hyperchromatic and polychromatic macrocytes were noted There were 5 normoblasts per hundred leukocytes There were 40,000 platelets

Gross Postmortem Examination—Petechial hemorrhages in the skin, epicardium, endocardium, gastric mucosa, urinary bladder and renal pelvis and moderate hyperplasia of the spleen (weight, 410 Gm, size, 19 by 10 by 5.5 cm) were noted. The liver weighed 1,660 Gm, the heart, 310 Gm. Hemosiderosis of the spleen, liver, bone marrow and lymph nodes, slight hyperplasia of the peripheral, thoracic and abdominal lymph nodes (greatest diameter, 15 mm) and confluent bronchopneumonia in the lower lobe of the left lung were noted.

Histologic Postmortem Examination—The bone marrow (femur) was rich in red blood corpuscles which were found chiefly outside the capillaries and sinusoids. The capillaries and sinusoids were collapsed, and fat cells were numerous. The reticular cells were prominent and contained many red blood cells. Immature blood cells occupied about 19.6 per cent of the marrow. A differential count showed 17 per cent undifferentiated lymphoid cells, from three to five times the size of small lymphocytes with a distinct rim of homogeneous basophilic cytoplasm that gave a negative reaction for oxidase and contained round nuclei with finely reticulated chromatin of varying density. The count also showed myeloblasts, 0.3 per cent, neutrophilic myelocytes, 19.8 per cent, neutrophilic leukocytes, 6.6 per cent, eosinophilic myelocytes, 0.5 per cent, eosinophilic leukocytes, 0.2 per cent, erythrogonia, 9.3 per cent, erythroblasts, 8.1 per cent, normoblasts (some of them very large with bizarre-shaped, pyknotic nuclei), 34.9 per cent, lymphocytes, 0.8 per cent, plasma cells, 0.2 per cent, monocytoïd cells, 1.6 per cent, megakaryoblasts, 0.1 per cent and megakaryocytes, 0.5 per cent.

The spleen showed extensive myeloid metaplasia of the pulp, with numerous myeloblasts, neutrophilic and oxyphilic myelocytes, many erythroblasts and normoblasts and single megakaryocytes. The malpighian bodies were much reduced in size. In the liver a moderate number of myeloblasts, myelocytes and erythroblasts were observed in the lumen of the sinusoids and in the periportal tissue. There were also a few megakaryocytes. The Kupffer cells were actively phagocytic. All the lymph nodes revealed advanced myeloid metaplasia, and nests of myeloid cells were also present in the fat tissue about the lymph nodes. The medulla and the cords between the cortical nodules were composed of myeloblasts, myelocytes, erythroblasts, normoblasts and single megakaryocytes. There was a great deal of erythrophagocytosis by the proliferated reticular endothelium of the sinusoids. The tracheobronchial and, in particular, the abdominal, lymph nodes contained also nests of undifferentiated lymphoid cells, which often formed a syncytium. These nests sometimes revealed intimate relations to the cytoplasmatic reticulum of the node. The thyroid gland, adrenal glands, pancreas, kidneys, heart and gastro-intestinal tract showed no significant changes.

The reaction for iron was as follows: Kupffer cells, + + +, hepatic cells, 0, splenic pulp, + + +, malpighian bodies, \pm , lymph nodes, + + +, bone marrow, + + +, and kidney, +.

CASE 3—History and Course—For the past seven months a Negro boy aged 11 years had been suffering from remittant pains in the hips, occasionally also of the knees and shoulders. The pains lasted for about two weeks and confined him to bed. On the day of his admittance to the hospital severe epistaxis had occurred. The boy had whooping cough and measles in infancy. There was unexplained fever for two weeks two years previously, and acute tonsillopharyngitis developed two months prior to admission.

The child was very pale and weak. The nose was filled with crusted blood, and he vomited everything he ate. The temperature was 98, the pulse rate 128

and the respiratory rate 24 The spleen was not palpable, and the peripheral lymph nodes were not enlarged

The Wassermann and Kahn reactions were negative Two blood cultures gave negative results The urine showed a trace of albumin The stools were normal Chemical analysis of the blood showed urea nitrogen, 28.45 mg, creatinine, 1.96 mg, and sugar, 106 mg, per hundred cubic centimeters Roentgen examination showed osteoporotic changes in both tibiae and slight sclerosis of the ilium but no changes in the skull

Three days after admission a blood transfusion was given Five days later the temperature rose to 103 F The next day a second transfusion was given Lymph nodes ranging in size from that of a pea to that of a filbert could then be palpated in both axillae and both groins

A lymph node was removed from the right groin for biopsy, and the examination showed hyperplasia, the tissue between the secondary nodules of the cortex and the cords of the medulla being infiltrated by undifferentiated lymphoid cells, larger than lymphocytes, with finely reticulated nuclei and scanty cytoplasm, and by single megakaryocytes The picture was suggestive of an early stage of hemocytoblastic leukemia

A few days later a serpiginous ulcer developed at the right side of the mouth and gradually spread to the right cheek By the seventeenth day the spleen was distinctly palpable at the costal arch, having increased rapidly in size The temperature varied between 101 and 102 F Roentgen treatment was given to the upper mediastinum The child died four weeks after admission to the hospital and eighteen days after the last blood transfusion

Examination of the Blood—Blood counts were made every third day On the day of admission the erythrocytes numbered 1,330,000, the hemoglobin content (Sahli) was 35 per cent and the leukocyte count was 14,550 During the following weeks the number of erythrocytes gradually fell to 600,000, the hemoglobin content to 15 per cent and the leukocyte count to 1,800 There were from 30 to 44 per cent undifferentiated cells (hemocytoblasts) These cells were two or three times the size of small lymphocytes The nucleus was round, with a deep, narrow indentation, which became marked toward the end of the illness The nucleus contained a dense, fine net of small chromatin granules which obscured the nucleoli The cytoplasm was narrow, it stained bright blue with Wright's stain and showed a negative reaction for oxidase At times 4 per cent of the cells showed mitotic division of the nucleus with about twenty-four chromosomes There were from 5 to 44 per cent neutrophilic leukocytes, the rest being small lymphocytes There were marked anisocytosis and poikilocytosis, very few polychromatophils, an average of 0.2 per cent of reticulocytes and 170,000 platelets The bleeding time was six minutes, the coagulation time, four minutes

Gross Postmortem Examination—Examination showed gangrenous stomatitis, osteoporosis, and petechial hemorrhages in the conjunctiva of the right eye, epicardium, gastric mucosa, Peyer's patches, kidneys and renal pelvis There was slight hyperplasia of the spleen (120 Gm) The liver weighed 920 Gm Fatty degeneration of the myocardium and moderate enlargement of the peripheral, thoracic and abdominal lymph nodes (greatest diameter, 20 mm) were noted

Histologic Postmortem Examination—The cortex of the long bones was unusually thin, and the haversian canals were very wide The trabeculae of the spongiosa were far apart and slender In the skull the diploe was compact, and the marrow spaces were narrow The bone marrow was extremely hemorrhagic (fig 1), looking like a lake of blood crossed by an occasional compressed capillary

Here and there a small nest of undifferentiated lymphoid cells and single normoblasts, neutrophilic myelocytes and megakaryocytes were found. The reticular cells were swollen and contained many engulfed erythrocytes. Similarly, the pulp of the spleen was greatly congested with blood. About the sheathed arteries, the trabeculae and the malpighian bodies there were accumulations of undifferentiated lymphoid cells which gave a negative reaction for oxidase and were often so densely grouped together that they seemed to form a syncytium. Bordering at the pulp, myeloblasts, hematogonia, erythroblasts, normoblasts, neutrophilic and oxyphilic myelocytes and plasma cells were found between the lymphoid cells. The undifferentiated cells were distinctly different from the small lymphocytes of the malpighian bodies. Many of the reticular cells of the pulp were filled with

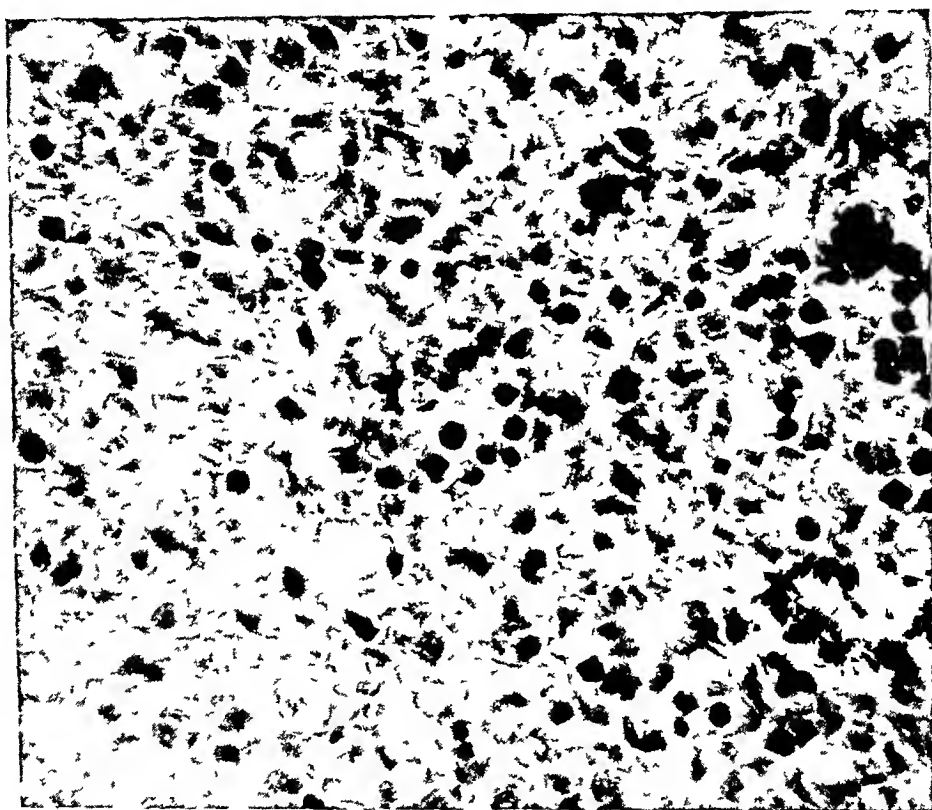


Fig. 1 (case 3)—Hemorrhagic bone marrow ($\times 600$)

erythrocytes and nuclear debris. The portal sinusoids of the liver contained a moderate number of undifferentiated lymphoid cells, myeloblasts, normoblasts, neutrophilic myelocytes and a few mature neutrophilic leukocytes. The periportal tissue was densely infiltrated by the cells described as present in the lumens of the sinusoids. The Kupffer cells were prominent and contained erythrocytes and nucleated cells.

In the lymph nodes the enormous erythrophagocytosis by the proliferated endothelial cells of the sinuses was striking (fig. 2). The cortex and medulla were rich in undifferentiated lymphoid cells, and there were also many myeloblasts and normoblasts and a few neutrophilic and oxyphilic myelocytes and large plasma cells. The para-aortic nodes contained many megakaryocytes. In the mediastinal and mesenteric nodes the cortical secondary nodules were well preserved and

showed histiocytic centers. About the larger vessels of the kidney, especially at the border of the cortex and the medulla, there were dense accumulations of undifferentiated cells which extended between the adjacent tubules. They were mixed with plasma cells, normoblasts and neutrophilic myelocytes.

The reaction for iron was as follows: Kupffer cells, +, hepatic cells, \pm , spleen, +++, lymph nodes, +++, bone marrow, +, and kidney, 0.

CASE 4—History and Course—The patient was a white man, aged 30, who had been employed as a color mixer in a soap factory. He stated that he was well until eleven weeks prior to admission, when a sharp, shooting pain developed at the tip of the spine and radiated toward the shoulders. At times there was pain in all the joints. He had lost a good deal of weight and felt exceedingly weak. One month prior to admission he began to cough up blood and to bleed from the gums.

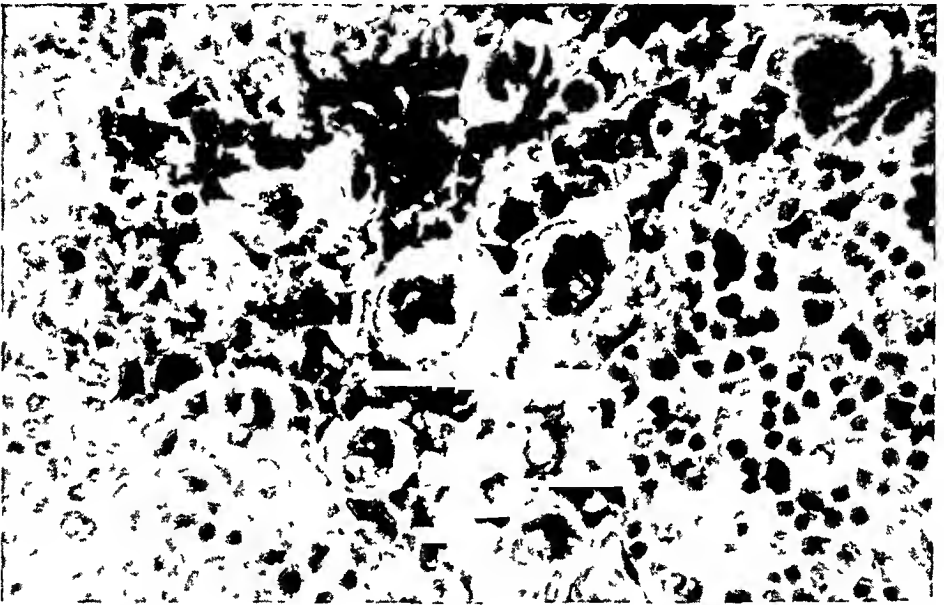


Fig 2 (case 3)—Erythrophagocytosis by the sinus endothelium of an iliac lymph node ($\times 600$)

On admission the patient was very pale. The posterior cervical and the axillary lymph nodes were slightly enlarged, discrete and firm. The heart and lungs were normal. The lower border of the liver was 2 fingerbreadths below the costal arch, and the lower pole of the spleen could be easily palpated. The spleen felt firm. There was a moderate enlargement of the epitrochlear and inguinal lymph nodes.

The icterus index was 3.5. The urine was normal. The temperature was 98.4 F.

A biopsy specimen from an inguinal lymph node revealed marked myeloid metaplasia with predominance of very immature hemocytoblastic cells.

During his stay in the hospital the patient became more anemic, and purpuric spots appeared over the extremities. A systolic murmur became audible over the apex of the heart. Two weeks prior to death he complained of failing vision, and an ophthalmoscopic examination disclosed retinal hemorrhages. He bled from the mouth and nose and died three weeks after admission to the hospital.

Examination of the Blood—On the day of admission the blood showed hemoglobin (Sahli), 30 per cent, erythrocytes, 1,920,000, and leukocytes, 4,750. The undifferentiated cells, which predominated, varied greatly in size, the smallest forms being slightly larger than small lymphocytes, while the largest cells reached a diameter of 30 microns. They were round or short oval, occasionally with semi-spherical, pseudopodia-like protrusions. The cytoplasm was scanty and basophilic and often contained several small vacuoles. The reaction for oxidase was negative. The nucleus occupied from two thirds to four fifths of the cell body and was round or oval with small indentations. The chromatin structure was densely reticular, and no nucleoli were seen. A differential count showed undifferentiated cells, 84 per cent, small lymphocytes, 8 per cent, neutrophilic leukocytes, 4 per cent, monocytes, 4 per cent, and blood platelets, 14,000. During the course of the illness the hemoglobin value decreased to 20 per cent, and the number of erythrocytes, to 1,140,000. The number of leukocytes fluctuated between 4,000 and 22,000 and shortly before death reached 5,100. The undifferentiated cells increased to 90 per cent.

Gross Postmortem Examination—Petechial hemorrhages were present in the mucosa of the lips, stomach and small intestine, the epicardium, endocardium and myocardium and the mucosa of the urinary bladder. The organs were severely anemic. There was marked hyperplasia of the spleen (weight, 1,015 Gm., size, 23 by 13 by 8 cm.). The liver weighed 2,550 Gm. There were moderate generalized lymphadenopathy, the largest nodes having a diameter of 30 mm., fatty changes of the myocardium, centrilobular focal necrosis of the liver, hemosiderosis of the liver and spleen and an old anemic infarct in the lower lobe of the right lung.

Histologic Postmortem Examination—The bone marrow (femur and rib) was composed of fat tissue with many focal, extravascular accumulations of blood. There were small islands of cells, occupying about 20 per cent of the marrow, which were formed by round cells the scanty cytoplasm of which was free from oxidase granules and often revealed small vacuoles. The nuclei contained a dense, fine net of small chromatin granules. In some of the cells the nuclei were indented, and the indentation was associated with a more compact chromatin structure. Occasionally, a nucleus was divided into two or three lobules. There were a few orthochromatic normoblasts, but no granulated cells were observed. In the pulp of the spleen, in the periportal tissue of the liver, extending into the adjacent portions of the lobules, in the peripheral, thoracic and abdominal lymph nodes and in the stroma of the kidneys, there were dense accumulations of cells similar to those noted in the bone marrow and typical of the condition. The nuclei showed the same indentations and segmentations, and the cells could be well separated from the lymphocytes. The reaction for oxidase was negative. In the lymph nodes many mitotic figures were seen. The portal sinusoids of the liver contained many typical cells. Layers of typical cells were found underneath the endothelium of the splenic, portal and pulmonary veins (fig. 3). Small groups of typical cells were also present in the interstitial tissue of the myocardium and about the lymphatic tissue of the intestinal tract. The arteries and veins leading to the infarct of the lung were occluded by organized thrombi, and in the granulation tissue filling the lumen there were groups of the undifferentiated round cells. In addition to the typical cells a moderate number of normoblasts and neutrophilic and oxyphilic myelocytes were observed in the splenic pulp, the liver and the lymph nodes. The inner zone of the adrenal cortex contained groups of normoblasts. A striking feature was the large number of plasma cells in all the hematopoietic organs. These cells reached a large size, and many of them had two nuclei.

The reaction for iron was as follows Kupffer cells, +++, hepatic cells, +++, spleen, +++, lymph nodes, +, kidney, \pm , and bone marrow, 0

CASE 5—*History and Course*—At the age of 5 years the patient, a white girl, had slight swelling and redness of the nose, this disappeared rapidly but recurred a year later. It again disappeared without treatment after a short time. Several months later it recurred again and then continued to grow slowly until it reached the size of a small egg. It closed the right eye and pushed the nose toward the left and the right side of the mouth downward. The swelling was firm, with a smooth, shiny, purple-brown surface. The surrounding skin was unchanged. Except for that lesion the child seemed to be in perfect health. A specimen was taken for biopsy. Ten radium treatments were given in the course of eight months.

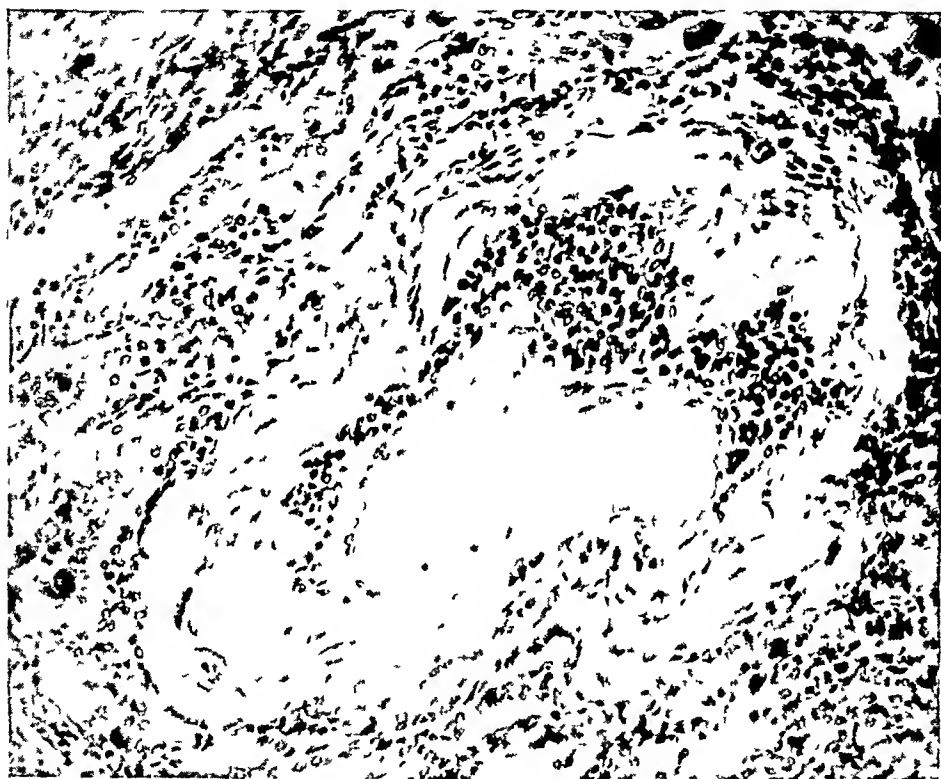


Fig 3 (case 4) —Subendothelial proliferation of undifferentiated hematic cells (hemocytoblasts) in a small pulmonary vein ($\times 300$)

The swelling disappeared gradually, and two months later there was only a small scar from the wound made in removal of the tissue for biopsy. Ten months later the child, then 8 years of age, had bloody diarrhea. The condition improved slightly, but the child felt extremely weak and feverish and complained of pains in the ears and of a cough. One week later she was admitted to the hospital.

On admission the child was pale. The temperature was 104.4 F, the pulse rate 154 and the respiratory rate 28. The drum of the left ear was deeply injected and thickened. The tonsils were enlarged and cryptic, and on the left side of the neck small, discrete lymph nodes could be felt. Fine, subcrepitant rales and bronchovesicular breathing were audible over the left lung posteriorly, and a loud, blowing systolic murmur was heard over the apex of the heart. The liver extended 2 fingerbreadths below the costal arch, and there was tenderness on deep palpation.

in the region of the spleen and the left kidney. The right axillary and both inguinal lymph nodes were enlarged and tender.

The urine was normal.

A blood transfusion was given, but the child died four days after admission.

Biopsy—The epidermis was thin, and the rete pegs were flattened (fig. 4). The reticular layer of the cutis and the subcutis were densely infiltrated by round cells, and these infiltrations were sharply separated from the epidermis by a layer of intact dermis. The infiltrations surrounded the hair follicles and sweat glands without invading or disfiguring them. They were composed of medium-sized round cells of even size and structure with a scanty cytoplasm and round or oval nuclei. The nuclei were rich in chromatin, which formed small granules and was evenly distributed. Some of the nuclei were pyknotic and crenated. There were single cells which were much larger than the average cells and possessed a lobulated nucleus. There were numerous mitotic figures. The cells were embedded in a delicate reticulum. In places where the infiltrations were less compact, strands of hyalinized collagenous fibers were seen between them. At the periphery of the infiltrations small groups of plasma cells were seen. The diagnosis was leukemia cutis.

Examination of the Blood—A blood count showed hemoglobin, (Sahli) 17 per cent, erythrocytes, 650 000, leukocytes, 1,600, lymphocytes, 99 per cent, neutrophils, 1 per cent, and platelets, 350,000.

Gross Postmortem Examination—The patient was severely anemic. There were petechial hemorrhages in the epicardium and endocardium, extensive hemorrhages in the mucosa of the colon, hemosiderosis of the liver, spleen, lymph nodes and bone marrow, severe fatty degeneration of the myocardium, fatty changes and centrilobular focal necrosis of the liver, tarry intestinal content, bronchopneumonia in the lower lobe of each lung, and a small scar on the right cheek. Cultures of the spleen and of blood from the heart were sterile.

Histologic Postmortem Examination—The bone marrow of the femur and of the vertebral bodies was composed of a fine, loose reticulum the meshes of which contained red blood cells and single immature red cells and granulocytes. The erythrocytes varied considerably in size and in hemoglobin content, and many of them were pale. The nucleated erythrocytes showed similar variations. There was a bizarre and abnormal segmentation of the nuclei of the normoblasts, and the few erythroblasts also revealed segmentation of the nuclei. The granulation of the myelocytes was poorly preserved, and the granules were often transformed into droplets. Many large, free histiocytes were present, and their cytoplasm was filled with iron granules or stained diffusely blue in the sections tested for iron. The myeloid elements occupied about 6 per cent of the marrow. A differential count showed neutrophilic myelocytes, 12 per cent, eosinophilic leukocytes, 1.6 per cent, erythroblasts, 4.8 per cent, normoblasts, 73.6 per cent, lymphocytes, 2 per cent, plasma cells, 2.4 per cent, and megakaryocytes, very scanty. The nuclei were pyknotic and the cytoplasm, homogeneous.

The splenic pulp was much congested with blood and rich in myeloid elements, among which normoblasts and neutrophilic and oxyphilic myelocytes predominated. While the granulation of the oxyphilic myelocytes stained well, that of the neutrophilic myelocytes was often indistinct. There were many plasma cells and single erythroblasts, myeloblasts and megakaryocytes. The portal sinusoids of the liver were filled with normoblasts, neutrophilic and oxyphilic myelocytes and myeloblasts. The myeloblasts formed small nests which were intimately connected with the cytoplasmatic reticulum formed by the Kupffer cells. The periportal

tissue was infiltrated by lymphocytes and contained also accumulations of myeloid cells. In the lymph nodes the sinuses were greatly widened and were filled by proliferated reticular endothelial cells which displaced active erythrophagocytosis. The sinuses as well as the cords of the medulla contained also neutrophilic and oxyphilic myelocytes, normoblasts and megakaryocytes. In the medullary cords of the para-aortic lymph nodes small nests of undifferentiated round cells were present which could be plainly distinguished from the lymphocytic cells and usually formed syncytial aggregates. In the myocardium the interstitial tissue was more cellular than normal owing to an increase in the myelocytes. In the fat tissue about the adrenal glands nests of mononuclear cells with ample cytoplasm were observed.

The reaction for iron was as follows: Kupffer cells, + + +, hepatic cells, \pm , spleen, + + +, bone marrow, + +, lymph nodes, + +, and kidney, 0.

COMMENT

In the five cases described there were many significant similarities. In each case the illness was of relatively short duration, and there was extremely severe anemia with a hemorrhagic diathesis. The autopsy observations in four cases were not suggestive of leukemia, although in three of these cases the blood picture was definitely leukemic. The essential macroscopic observations were those of severe anemia and of an excessive destruction of blood, as indicated by the rusty discoloration of the liver, spleen and lymph nodes. Microscopically, the extensive destruction of the erythrocytes was one of the most striking features. In four cases the reticulohistiocytic cells of the blood-forming organs were packed with erythrocytes, and in all five cases there was marked hemosiderosis of the organs of the reticulohistiocytic system. Since erythrophagocytosis and hemosiderosis were present also in the cases in which blood transfusions had not been given the excessive destruction of blood could not be related to the transfusions. Phagocytosis and disintegration of the red blood cells were also observed in lymph nodes that drained from areas which were not the site of hemorrhages. The excessive destruction of blood was associated with an increased reactive myelopoiesis which occurred chiefly outside the bone marrow. This extramedullary myelopoiesis was orderly and showed a distinct tendency to produce mature blood cells, not being different from the extramedullary formation of blood cells often observed in cases of severe anemia. The condition of the bone marrow was of great interest since it was different from the condition which is usually observed in cases of acute leukemia. The bone marrow was engorged with erythrocytes and revealed very little evidence of activity. In none of the five cases did the myelopoietic tissue occupy more than 20 per cent of the marrow spaces.

Recently puncture of the bone marrow has come into vogue, and great significance has been attributed to the examination of material obtained from the sternal marrow either by surgical removal or by

aspiration If, for instance, in case 1, in which there was a distinctly leukemic blood picture, the sternal marrow had been examined *in vivo*, the result would have been disappointing In case 2, in which the diagnosis was clinically obscure sternal puncture probably would have been of little help in determining the correct diagnosis

In addition to the typical reactive myelopoiesis in all five cases a proliferation of very immature blood cells was noted which varied in extent, being insignificant in case 5 and most marked in case 4 That type of proliferation of immature blood cells and their differentiation from the mesenchyme throughout the body occurs only in cases of leukemia There is still much controversy as to the type of cell observed in certain cases of acute leukemia The majority of investigators agree that in addition to myeloblastic myelocytic, lymphoblastic, lymphocytic, monocytic and plasmacellular leukemia there are forms of leukemia which defy classification because the cells are so immature that they cannot be identified with any of the known precursors of white or red blood cells There is growing evidence in favor of considering these very immature cells as the common parental cells of all blood cells In the German literature the term stem cell leukemia (Hoff¹⁸) has been widely adopted, while Italian authors, under the influence of Ferrata, speak of hemocytoblastic leukemia (Marziani,¹⁹ Debiasi,²⁰ Callerio,²¹ Samek,²² Simonetti²³ and many others) In this country both terms—hemocytoblastic leukemia and stem cell leukemia—are being used Concerning the morphology of the hemocytoblast the reader is referred to the description given in the case reports The hemocytoblast is a cell which gives a negative reaction for oxidase and which is distinctly different from the lymphoblast, monoblast, myeloblast, megakaryoblast and erythrogonium (proerythroblast, megaloblast) Showers of hemocytoblasts may enter the blood in any type of case of acute leukemia, and even in cases of subacute or chronic leukemia there may be a transient hemocytoblastic stage In the earlier literature, published before the hemocytoblast had been recognized the appearance of hemocytoblasts in a case of myelogenous leukemia was interpreted as a shift from the myeloid to the lymphatic side (mixed cell leukemia) Hemocytoblastic leukemia may take a leukemic, subleukemic or aleukemic course and in my experience has been the most common form of pure acute leukemia, the lymphatic and myelogenous form frequently being the terminal stage of chronic leukemia

18 Hoff, F Virchows Archiv f path Anat **261**:142, 1926

19 Marziani, R Arch ital di anat e istol pat **1** 1015, 1930

20 Debiasi, E Haematologica **12** 719, 1931

21 Callerio, G Haematologica **13** 49, 1932

22 Samek, E Haematologica **14** 37, 1933

23 Simonetti, R Riv di clin pediat **30** 161, 1932

In three of my cases the hemocytoblast was found in the blood. The leukocyte count varied between 1,800 and 33,800. In other cases of hemocytoblastic leukemia which are not included in this report I have encountered leukocyte counts up to 700,000. In two cases there were also young granulocytes and precursors of erythrocytes in the blood smears, and in one case the granulocytes showed severe toxic alterations. In four cases the number of platelets was moderately to markedly diminished, while in one case it was within normal limits.

Case 5 is of particular interest. Three years prior to death a recurrent cutaneous lesion of the face developed which disappeared spontaneously twice within two years. With the third recurrence the lesion continued to develop and receded promptly under radium treatment. The child was in good health for ten months after the treatment had been completed. The clinical and histologic picture of the third recurrence was characteristic of the tumor-like form of specific leukemia cutis. I refer to the location at the root of the nose, to the sharp demarcation of the tumor-like lesion from the surrounding normal skin, to the arrangement of the infiltrating cells about the hair follicles and the sweat glands and to the distinct demarcation of the infiltrations from the subpapillary layer of the cutis which was unchanged while the cells extended deep into the subcutaneous tissue (fig. 4, Arndt²⁴ Gans,²⁵ Patrassi²⁶ and others). It is chiefly in the lymphatic form of leukemia cutis that the face is involved, especially the region about the nose (Gans,²⁵ Kreibich,²⁷ Hirschfeld¹⁶ Arzt²⁸), and Pinkus²⁹ therefore spoke of the "acriolesions" in cases of lymphatic leukemia of the skin. From the classic form of lymphadenosis cutis the picture in my case differed slightly. The infiltrations were more pleomorphic, and the cells were larger than those that usually are found in lymphatic-leukemic infiltrations of the skin. Unfortunately I have no information in that case as to the blood picture during the stage of isolated leukemia cutis. There was, however, nothing in the clinical picture to suggest a blood dyscrasia.

Cases of isolated leukemia of the skin have been repeatedly described (Zumbusch,³⁰ Pinkus,²⁹ Roessle³¹), and even at autopsy the leukemic

24 Arndt, cited by Arzt²⁸

25 Gans, O. *Histologie der Hautkrankheiten*, Berlin, Julius Springer, 1925, vol. 1, p. 575.

26 Patrassi, G. *Folia haemat* **50** 415, 1933.

27 Kreibich, K. *Arch f Dermat u Syph* **47** 185, 1899.

28 Arzt, L. *Wien klin Wchnschr* **46** 1125, 1933.

29 Pinkus, F. *Arch f Dermat u Syph* **50** 37, 1899.

30 Zumbusch, cited by Roessle³¹

31 Roessle, R. *Virchows Arch f path Anat* **275** 310, 1930.

process may be found to be almost exclusively restricted to the skin (Roessle³¹) In this connection the case described by Dragisic³² may be cited The patient, a girl aged 11 years, suffered from an aleukemic, leukopenic lymphadenosis, which after five months terminated in acute leukemia Following roentgen treatment of the enlarged lymph nodes leukemic infiltrations of the skin developed Later the infiltrations of the skin disappeared, while the lymph nodes enlarged again

During the short final illness in my case there were very severe anemia and leukopenia with extreme neutropenia The normal platelet



Fig 4 (case 5)—Section of biopsy material from the skin, showing the arrangement of the infiltrations about the hair follicles and the sharp demarcation of the infiltrations from the subpapillary layer of the cutis, the stretching of the epidermis and the extension of the infiltrations into the subcutis ($\times 150$)

count spoke against a diagnosis of aplastic anemia or hemorrhagic aleukia Anatomically the leukemic changes were very insignificant and consisted of a focal proliferation of very immature hematic cells in the medullary cords of the para-aortic lymph nodes By comparing the changes in the para-aortic lymph nodes with those seen in cases of typical hemocytoblastic leukemia, one can readily recognize that the

32 Dragisic, B *Wien klin Wchnschr* 45 1165, 1932

differences were merely quantitative and not qualitative. The chief argument in favor of the diagnosis of very early acute leukemia was the preceding tumor-like leukemic infiltration of the skin of the face. I am therefore of the opinion that the case was one of isolated leukemia cutis in which almost one year after the leukemic lesion of the skin had been treated successfully with radium death occurred in the initial anemic stage of acute leukemia. Because of the long interval between the radium treatment and the onset of anemia, causal relations between the two can be excluded.

CONCLUSIONS

The early stages of a disease offer the best possibility to study the pathogenesis, since in the later stages the changes may have advanced so far that they obscure the underlying pathologic process. Clinical and experimental observations have repeatedly suggested that leukemia may be related to or follow an abnormal destruction of blood. Thus leukemia has been observed in persons exposed to benzene, arsenicals, radium and other substances known to cause destruction of the blood cells (Emile-Weil³³, Delore and Bergomano³⁴, Falconer³⁵, Vaughan, Terplan and Sanes,³⁶ and others). Leukemia has been produced in mice by injections of benzene (Lignac³⁷) or indole (Bungeler³⁸), and particularly in Bungeler's experiments, the relations between a severe alteration of the blood cells and the leukemia is striking. The five cases which I have selected from a large series of cases of acute leukemia indicate that at least in some instances leukemia is preceded by an excessive destruction of blood cells and that the patient may succumb to the anemia when the leukemic changes are still too insignificant to account for the lack of normal blood cells. In one case there were also evidences of a grave alteration of the granulated white blood cells. As a compensation for the excessive destruction of blood, a reactive myelopoiesis has been observed. Comparing the different foci of myelopoiesis, one obtains the impression that under the continuous stimulation of the destruction of the blood cells an increasing number of immature precursors of blood cells are called into existence until the hemocytoblastic stage is reached. Here and there, the hemocytoblasts may reveal attempts at maturation, but rapid multiplication seems to prevent their differentiation.

33 Weil, P. E. *Presse med* **33** 1297, 1925, *Bull et mem Soc med d hop de Paris* **48** 193, 1932.

34 Delore and Bergomano. *J de med de Lyon* **9** 227, 1928.

35 Falconer, E. H. *Am J M Sc* **186** 353, 1933.

36 Vaughan, S. L., Terplan, K., and Sanes, S. *Arch Path* **18** 923, 1934.

37 Lignac, G. O. E. *Klin Wchnschr* **12** 109, 1933.

38 Bungeler, W. *Klin Wchnschr* **11** 1982, 1932. *Frankfurt Ztschr f Path* **44** 202, 1932.

It has been previously stated that several investigators have considered pathogenic relations between the aleukocytic conditions and the leukemia. I believe, however, that there are principal differences between the aleukocytic diseases and leukemia. Granted that in some cases of agranulocytosis, aplastic anemia and hemorrhagic aleukia the precursors of the blood cells may fail to mature because of the lack of a hypothetical maturation factor, the reverse of the formation of the blood cells to the hemocytoblastic stage occurs only in leukemia. It is also only in leukemia that throughout the body the mesenchyme acquires the potency to produce blood cells. In children particularly, agranulocytosis and aplastic anemia may lead to extramedullary myelopoiesis (Willi³⁹), which, however, is entirely different from the leukemic changes.

As to the causative agent of the initial anemia, my cases do not yield any definite information. Three of the cases were in children and two were in adults. One of the adults was employed as dye mixer in a soap factory, but I have not been able to obtain exact information as to the nature of the dyes which he had used. Except perhaps in case 3, there were no relations to infections. To the trauma in case 1 I do not attribute any great significance. I believe that the child was already sick when the trauma was sustained and that the injury may perhaps have precipitated some of the symptoms. A traumatic etiology of leukemia is very doubtful, and what has been said about the relations between trauma and cancer (Ewing⁴⁰) probably holds true also for leukemia.

SUMMARY

On the basis of a hematologic and histologic study of five cases of acute leukemia with very severe anemia which revealed a striking evidence of destruction of blood and a marked disproportion between the severity of the anemia and the extent of the leukemic changes, it is suggested that an initial abnormal destruction of blood cells may be of significance in the pathogenesis of acute leukemia.

39 Willi, H. *Jahrb f Kinderh* **142** 102, 1934

40 Ewing, J. *Arch Path* **19** 690, 1935

Case Reports

DEFICIENCY OSTEOPOROSIS

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There is clinical evidence that deficiency osteoporosis is due to lack of vitamin D and hence may be an adult form of rickets

The first cases were observed in Vienna in the autumn of 1918¹ and in the cities of South Germany shortly afterward² The ages of the patients varied from 15 to 80, while the sex distribution was the same as in the general population The symptoms came on gradually and were of several months to one and a half years' duration The patients complained of easy fatigability and pains in the ribs, sacrum and legs The gait was waddling Many patients showed deformity of the thorax and kyphoscoliosis of the thoracic spine Bowing of the extremities was also present, particularly in older persons Pelvic deformities were rare and were observed only in females All of the bones were sensitive to pressure Abductor spasms as well as positive Chvostek and Trousseau signs could be elicited in some cases Tetany was fairly common Edema of the lower extremities and anasarca were frequent Most patients, especially the older ones, were markedly emaciated Their diets consisted of potatoes, turnips, carrots, lean soups and war breads, proteins and animal fats were practically absent from their diets Spontaneous fractures and infractions were described by Eisler³ and Hass⁴ as characteristic

Partsch⁵ described fifteen cases of deficiency osteoporosis pathologically, and his findings agree in general with those in this case Looser⁶

1 Edelmann, A *Wien klin Wchnschr* **32** 82, 1919 *Wien med Wchnschr* **69** 800, 1919 Hahn, J *Wien klin Wchnschr* **32** 713, 1919 Porges, O *Wien med Wchnschr* **69** 801, 1919 Porges, O, and Wagner, R *Wien klin Wchnschr* **32** 385, 1919 Zak, E *Wien med Wchnschr* **69** 803, 1919

2 Alwens, W *Munchen med Wchnschr* **66** 1071, 1919 Fromme, A *Deutsche med Wchnschr* **45** 510, 1919 Geisler, A *Ueber das Krankheitsbild der "Hunger-Osteomalazie" bei Erwachsenen*, Breslau, M Bermann, 1919 Hamel, O *Deutsche med Wchnschr* **46** 68, 1920

3 Eisler, F *Wien med Wchnschr* **71** 482, 1919, *Fortschr a d Geb d Rontgenstrahlen* **30** 67, 1923

4 Hass, J *Wien klin Wchnschr* **32** 677, 1919

5 Partsch, F *Deutsche med Wchnschr* **45** 1130, 1919

6 Looser, E *Deutsche Ztschr f Chir* **152** 210, 1920, *Verhandl d deutsch path Gesellsch* **2** 281, 1927

claimed that late rickets and so-called juvenile osteomalacia are identical anatomically. In biopsies in his cases of rachitis tarda he found atrophy of the old bone, osteoid borders from 10 microns to more than 200 microns in width, a moderate number of osteoclasts and a fibrous marrow with a few small hemorrhages and lymphocytic foci. This picture, he claimed, is typical for rickets, and he regarded failure to calcify the osteoid as pathognomonic of rickets. The latter phenomenon was conspicuous in the case about to be described.

Miles and Feng⁷ made chemical studies of the blood in ten cases of osteomalacia in women in northern and western China. Clinically, these cases were similar to those of deficiency osteoporosis. The usual diet consisted of a vegetable preserved in brine, wheat flour products or millet and practically no animal protein or fat. The blood calcium and phosphorus ranged from 5.2 to 7.4 mg and from 1.8 to 3.8 mg per hundred cubic centimeters of blood respectively. When cod liver oil was added to the usual diet the patients showed a marked clinical improvement and a net gain in the blood calcium, but basic calcium phosphate made the patients worse.

Dalyell and Chick⁸ and Hume and Nirenstein⁹ suspected that lack of some accessory food factor in the diets of their Viennese patients is the etiologic agent of deficiency osteoporosis. One hundred grams of cod liver oil administered over a period of one week cured the average patient. Phosphorus combined with oil was effective only if the oil was cod liver oil. Various vegetable oils and fats like rape and olive oils were ineffective. Patients ill more than a year gave a history of a summer remission and a winter relapse. It appears, then, that the accessory food factor lacking is vitamin D.

REPORT OF A CASE

A woman, aged 64, a domestic servant, had menstruated regularly from the twelfth to the fiftieth year, she had had nine pregnancies, eight deliveries and one abortion, she had always been undernourished and in poor economic circumstances. In July 1920 she gave a history of pain of two years' duration in both knee joints and in the lower part of the right leg. The right shoulder joint was slightly ankylosed. She received phosphorus with no improvement.

In August 1922 there were pains in the right hip joint, but shortening of the femur was not demonstrable. In October the right greater trochanter was 5 cm above Nelaton's line with considerable shortening of the femur due to a lateral convex bowing in the region of the trochanters and the neck, the left greater trochanter was 1 cm above Nelaton's line. Percussion and compression over the right trochanter were painful. There was a kyphosis of the thoracic spine.

Sun baths diminished the pain, but she had to use a cane. Symptoms became worse and a spontaneous fracture of the neck of the right femur occurred in

7 Miles, L. M., and Feng, C. T. *J. Exper. Med.* **41** 137, 1925.

8 Dalyell, E. J., and Chick, H. *Lancet* **2** 842, 1921.

9 Hume, E. M., and Nirenstein, E. *Lancet* **2** 849, 1921.

January 1923 Phosphorus, 0.005 Gm three times a day, was without effect. In June 1924 the patient began to show evidences of cardiac decompensation and died the following August.

The diagnosis was osteoporosis, atrophic fracture of the neck of the right femur, heart failure and edema.

The autopsy showed severe anasarca. The right lower extremity was shortened and rotated strongly inward, and there was a right-angled bend in the femoral

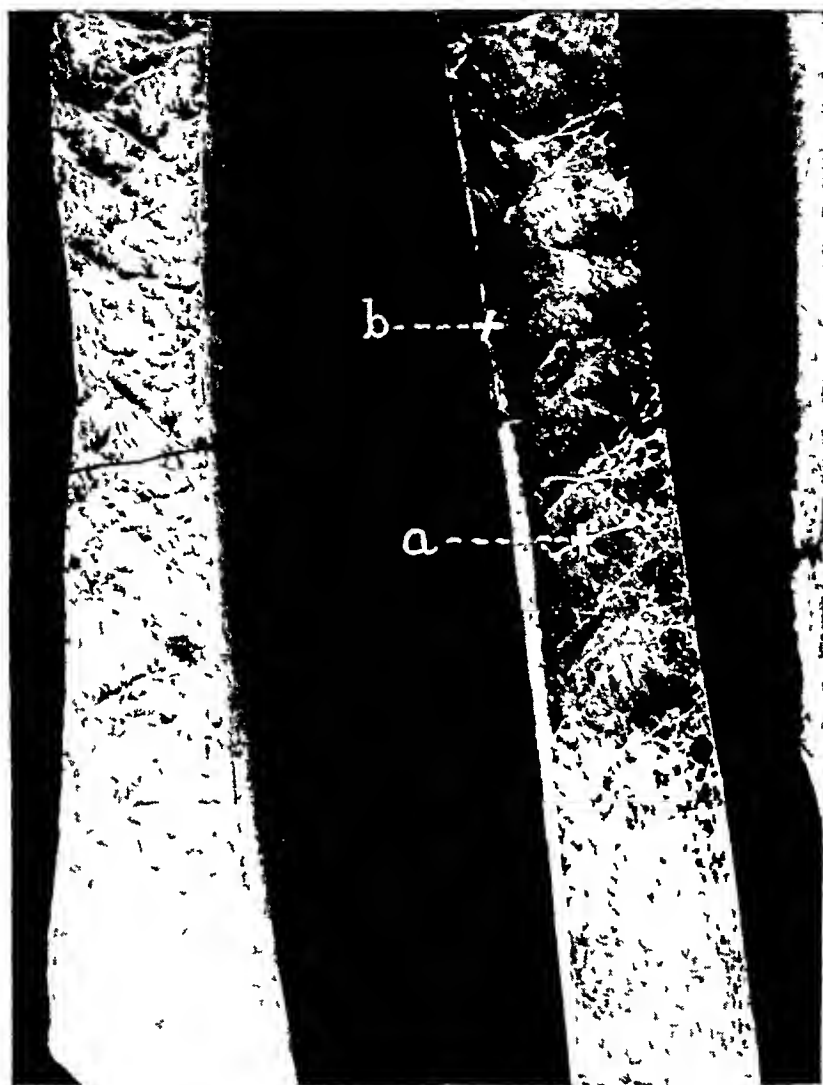


Fig. 1—Midshaft of the right femur, showing an extreme degree of porosis (*a*) disappearance of the spongiosa except for fine trabeculae, with resulting widening of the marrow cavity, (*b*) atrophy of the cortex.

shaft. Above the condyles, 5 cm from the joint surface, there was a recent complete slightly impacted fracture with a large circular subperiosteal osteophyte. The marrow cavity of the midshaft region was 3.4 cm wide and contained hyperemic fatty marrow. The cortex in this region was about 0.5 cm in maximum thickness and was made up of one or two separate paper-thin bony trabeculae. The spongiosa was sparse here (fig. 1). The kinked portion of the shaft, how-

ever, had a marrow cavity 1 cm wide, and the cortex on the convex side of the kink was 1.9 cm thick, this thickening having resulted from a callus with the structure of a soft, compressible, uncalcified spongiosa. The spongiosa at either end of the femur was extremely porotic.

The right tibia showed a subperiosteal fracture 3.5 cm above the ankle joint with widespread hemorrhage into the marrow.

The cartilages of the right knee and ankle joints in places showed almost complete defects which extended practically to the underlying bone. The right patellar cartilage had almost entirely disappeared.

The left femur was normal in shape. The cortex varied from 0.1 to 0.3 cm in thickness, its structure was that of a spongiosa consisting of thin parallel longitudinal trabeculae. The spongiosa of the condyles and head was very porotic. The marrow cavity at the midshaft was 2.1 cm wide.

The left tibia was not altered in shape. Its cortex was 0.2 cm thick and its marrow cavity markedly widened.

The pelvis was distorted, the symphysis pubis being pushed to the left. The lumbar spine was moderately bowed with a right scoliosis. The upper thoracic spine showed a strong arcuate kyphosis. The lateral diameter of the thorax was much reduced.

The skull was mesocephalic, the maximum thickness was about 0.6 cm. Neither the external nor the internal tables were recognizable. The calvarium also had the structure of a soft porotic spongiosa.

The upper extremities showed no deformities.

All the bones could be easily cut with the knife.

Microscopic Observations—The right femur, the right patella and the vault of the skull were examined microscopically.

The periosteum was of variable but normal thickness and, in the deeper layers of the cambium, showed lymphocytes and erythrocytes, solitary or in groups, as well as intracellular and extracellular blood pigment.

The extraordinarily severe generalized diminution of osseous tissue of the skeleton was especially marked in the lower two thirds of the femur (fig 2) and in the patella. Only exceptionally was it 2 and rarely 3 trabeculae thick. The cortex of the neck and subtrochanteric region of the femur was of normal thickness or even thicker than normal, this difference apparently representing a compensatory change to meet the mechanical stresses on the femur in this region (fig 3A). But even so the cortex here had the structure of a wide-meshed spongiosa formed by a few thin irregularly distributed trabeculae, which were most numerous beneath the periosteum. The marrow spaces between the trabeculae were filled chiefly with fibrous marrow, apparently representing additional efforts on the part of the body to meet the mechanical stresses in this region.

The high grade osteoporosis manifested itself also in the enormous number of lacunar resorption spaces found everywhere. The periosteal surface of the femur for long stretches was eaten away. Cortical and spongiosa trabeculae in all regions were extremely thin because of this lacunar resorption (fig 3B). Long and short interruptions (fig 2) in the cortex of the long bones were further evidence of this most severe bone atrophy. In contrast to Paget's disease and the degree of destruction here, osteoclasts were relatively rare.

Osteoid played a most important part in the skeletal structure, but its distribution was irregular (fig 3B). There were places where the osseous tissue consisted entirely or almost entirely of osteoid, other places where it was formed equally of osteoid and calcified bone, and still other places where, though the osteoid was comparatively diminished, nevertheless it was pathologically increased.

The points of greatest mechanical stress were best calcified—i. e., the outermost portions of the tubular bones—in agreement with the calcification law of Erdheim. The lower two thirds of the femoral shaft (fig 2) and the cortex of the anterior portion of the patella showed the least osteoid and the most calcified bone. Much osteoid was found in the trabeculae of the spongiosa-like cortex of the crooked portion of the femur, in lacunar resorption spaces in general and in haversian canals. There was little osteoid in the spongiosa because the spongiosa had practically disappeared.

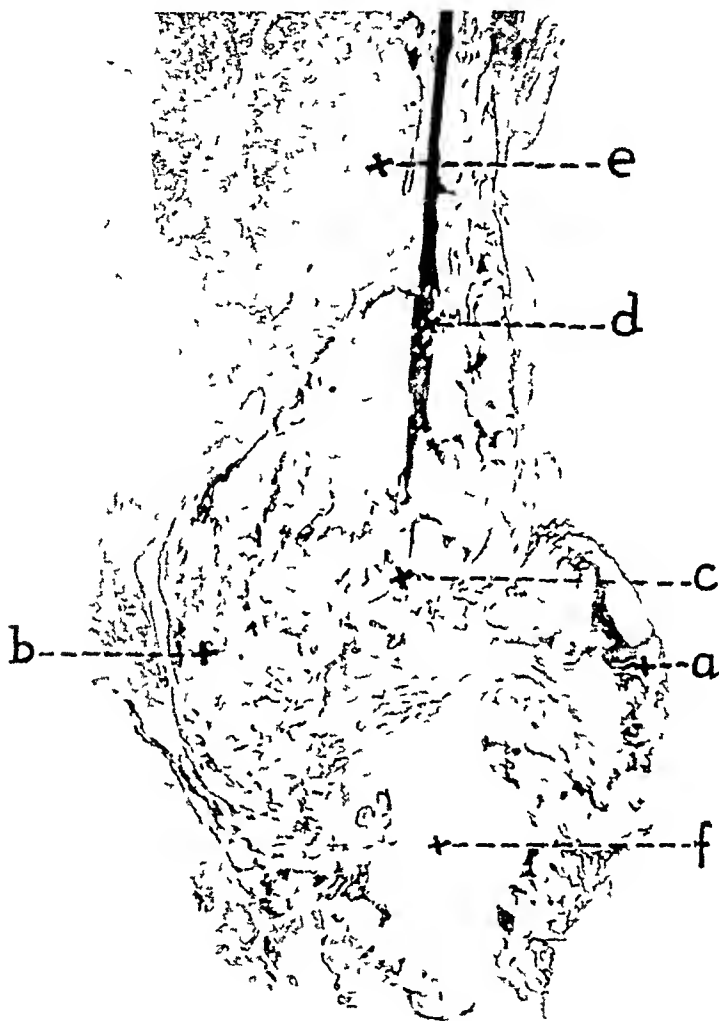


Fig 2—Subperiosteal fracture of the femoral shaft \times about 30. (a) periosteal callus, (b) endosteal callus, (c) intermediate callus, (d) extremely thin but well calcified cortex with interruptions in the cortical continuity, (e) disappearance of the spongiosa, (f) artefact—a pulling out of the lower end of the fracture fragment on sectioning.

The thickness of the osteoid varied greatly. Normally osteoid does not exceed 10 microns in thickness, according to Pommer¹⁰. Two thousand, seven hundred

¹⁰ Pommer, G. Zentralbl. f. Herz- u. Gefässkr. **16** 342, 1924, Arch. f. klin. Chir. **136** 1, 1925.



Fig 3—*A*, transverse section of the subtrochanteric region of the right femur, \times about 30 (a) external boundary sheath, (b) internal boundary sheath, (c) trabeculae spanning the space between the external and internal boundary sheaths and forming a thick wide-meshed porotic spongiosa at the site of the cortex, (d) marrow cavity

B, section from the subtrochanteric region, \times 100 (a) osteoid, lamellated in structure, (b) calcified bone, (c) cement line, (d) appositional resting line, (e) lacunar resorption of calcified bone, (f) micro-fracture, Y-shaped, with fibrinoid in the fracture space, (g) fibrous marrow between the trabeculae

and seventeen (2,717) measurements were made. The average values varied in different regions. In the femur, osteoid was thickest in the crooked portion and thinnest in the lower two thirds of the shaft. The average thickness was greater in the patella than in the femoral condyles and least in the calvarium. The minimum measurements in all regions examined were within normal limits.

The osteoid structure was not uniform. In some places it had a distinctly lamellated structure with few osteoblasts. In other places it was fibrillated, plexiform and rich in osteoblasts. And then, again, in many places it appeared to have a homogeneous structure. The boundary between mature and immature bone was a lacunar cement line, an appositional resting line or a wavy granular zone of Pommer (fig 3B). Osteoid was not always present as a border or margin on the surface of calcified bone. Many times the deeper or central parts of the osseous tissue remained uncalcified, while near the free surface there was a discontinuous calcification, a phenomenon often present in rickets. Partial calcification of osteoid was common. This sometimes took the form of small isolated dark blue granules of calcium within the osteoid, sometimes the process was homogeneous, the osteoid staining a pale blue indicating incomplete calcification.

Measurement of the Thickness of the Osteoid Tissue

Region	Measurements	Thickness, Microns		
		Average	Maximum	Minimum
Femoral head	41	24	80	8
Trochanteric region	479	49	272	4
Bowed portion of the upper part of femoral shaft	1,129	58	512	4
Straight portion of the lower part of femoral shaft	468	24	96	4
Femoral condyles	269	25	128	4
Patella	209	34	352	4
Calvarium	142	20	51	4
Total measurements	2,717			

The chondro-osseous junctions showed characteristic lesions. The old calcification zone and the old osseous junctional plate had been largely destroyed from below, the destructive process often extending into and beyond the transitional zone of the joint cartilage (fig 4). Later a new preparatory calcification zone might be formed on the new internal surface of the cartilage, but this new calcification zone was markedly advanced toward the free joint surface. In the most favorable instances the new preparatory calcification zone was covered by a new, though thin, poorly calcified, irregular, discontinuous osseous junctional plate. Yet even the new preparatory calcification zone might be absent so that the new osseous junctional plate and the uncalcified cartilage were in direct contact. The trabeculae of the underlying spongiosa inserted in the osseous junctional plate if one was present, in the absence of one, they inserted in the preparatory calcification zone, and if the latter was also absent, the trabeculae rested directly on uncalcified cartilage. At the edge of the joint the cortex joined the osseous junctional plate as is normal or the joint cartilage ended abruptly in a calcification zone into which the cortex inserted.

Destruction of the original joint cartilages examined (right femoral head, condyles and patella) was severe. In the patella, for example, there were only small rests of old cartilage. Rossi¹¹ and Rabson¹² have shown that disuse, of

11 Rossi, A. L. Virchows Arch f path Anat **284** 256, 1932
12 Rabson, S. M. Virchows Arch f path Anat **291** 624, 1933

which ankyllosis is the most complete form, causes marked destruction of the joint cartilage proper. This patient was bed-ridden over a period of years. The cartilage showed lacunar resorption, but it also showed incomplete destruction as described by Pommer, this change being preceded by the formation of Weichselbaum's spaces. The normal basophilic staining of the cartilage had been largely replaced by a red-violet stain, further evidence of damage. Pads of new cartilage (fig 4) were present in the indentations eaten out of the internal surface of the old joint cartilage or between the old cartilage and the new osseous junctional plate.

Fractures and infractions have been described, in regard to roentgen appearance,¹³ as characteristic of deficiency osteoporosis. There was a typical subperiosteal fracture without displacement in the lower portion of the femur.

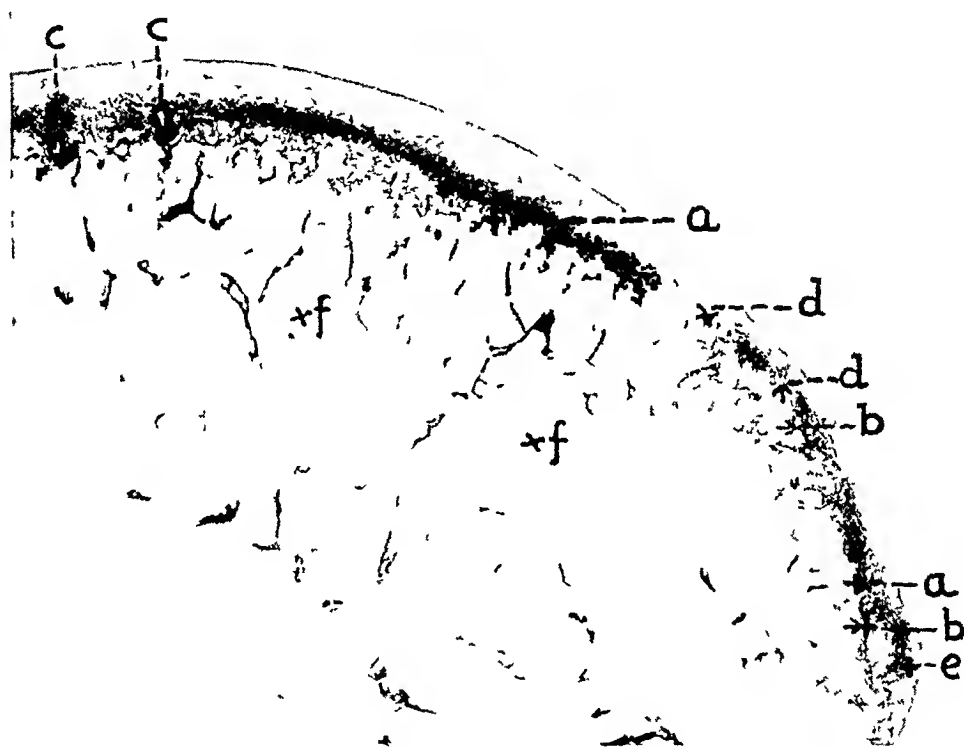


Fig 4—Section of the femoral head (\times about 30) showing the joint cartilage and subjacent spongiosa. (a) disappearance of the old calcification zone and osseous junctional plate, (b) new calcification zone and new osseous junctional plate, (c) pads of new cartilage between the new osseous junctional plate and the old cartilage, (d) deep indentations eaten out of the cartilage from below, extending up to and into the transitional zone, (e) hyperplasia of the old cartilage, (f) extreme porosity of the spongiosa of the femoral head, showing the short, thin, widely spaced and scanty trabeculae.

(fig 2) The periosteum and the bone marrow in the vicinity of the fracture were neither torn nor interrupted. The callus was ring-shaped with the upper and lower fragments inserted into it. It had three divisions—periosteal, endosteal

¹³ Kienbock, R. Fortschr. a. d. Geb. d. Röntgenstrahlen **33** 862, 1925.
Milkman, L. A. Am. J. Roentgenol. **24** 29, 1930. Eisler.³

and intermediate. Periosteally and endosteally it was bounded by a thin partially calcified shell of bone, within which there were a few thin, irregular trabeculae poorly calcified and in places cartilaginous, and fibroblasts, disseminated fat and marrow cells, leukocytes, blood pigment and a moderate number of necrotic areas.

Microfractures were also present (fig 3B). These showed more or less gaping fracture spaces, frequently Y-shaped, containing reddish fibrinoid, a sign that they were not artefacts. Doubtless the crook-shaped bend of the subtrochanteric region of the femur was the result of repeated microfractures which had healed. Small compact calluses consisting of a mass of primitive osteoid with isolated calcium deposits and mature hyaline cartilage were present in the bent

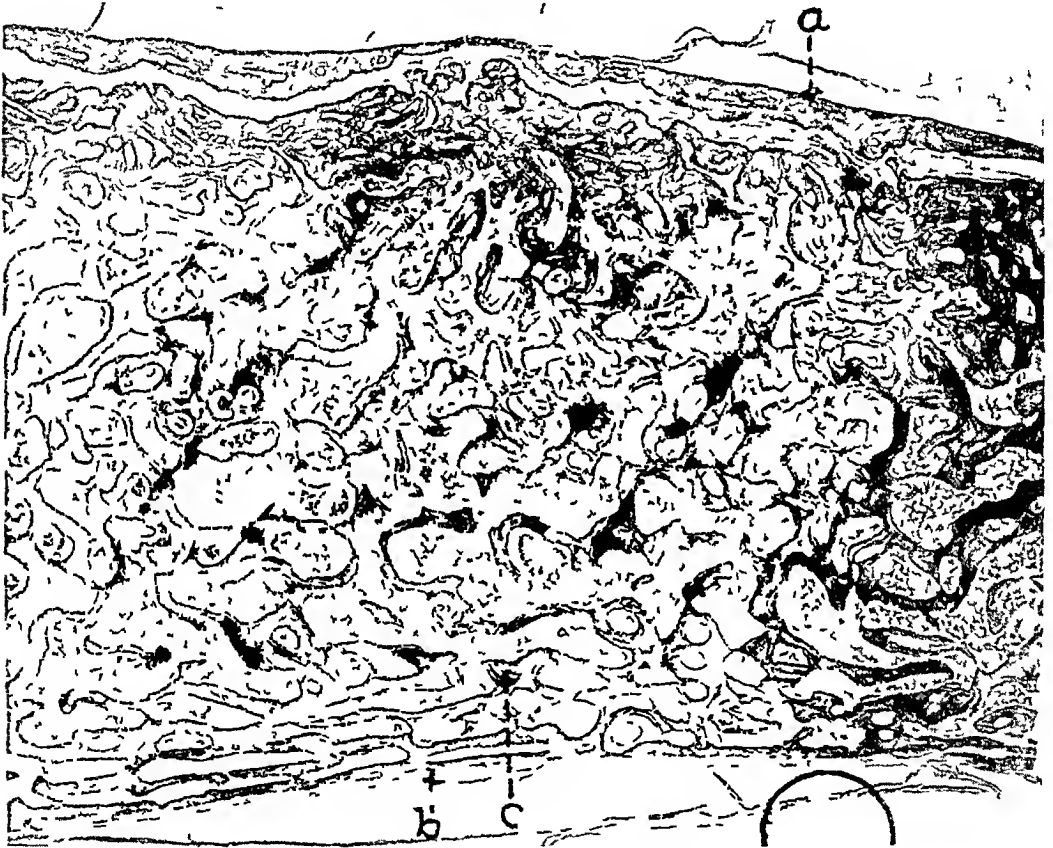


Fig 5—Section of the calvarium, \times about 30. (a) pericranial portion showing the disappearance of the external table and its replacement by a thin sheath of bone, (b) dural portion showing the replacement of the internal table by a thin sheath of bone, (c) trabeculae forming a spongiosa replacing the normal diploic structure.

portion of the femur and in the patella and were to be interpreted as the healing stages of microfractures.

The tables of the calvarium with the intermediate diploic structure, as was to be expected, were gone (fig 5), and the calvarium consisted of a spongiosa bounded durally and pericranially by thin sheaths of bone. At death, in spite of this remodeling, lacunar resorption was only moderate. The calcium content of the calvarium was much nearer normal than that of the femur, and osteoid was present

only in moderate amount (table) All in all, however, in comparison with the normal this calvarium was obviously atrophic

Unfortunately the parathyroids were mislaid and hence were not available for microscopic study

SUMMARY

A case of deficiency osteoporosis is described in a 64 year old white woman Clinically, deficiency osteoporosis appears to be due to lack of vitamin D The changes at the chondro-osseous junctions in deficiency osteoporosis are similar to those in rickets Osteoid is markedly increased in amount and thickness as it is also in rickets The last two changes are considered as pathognomonic of rickets Hence there is some histologic evidence that deficiency osteoporosis and rickets may be the same entity

RETICULO-ENDOTHELIAL SARCOMA OF THE SPLEEN

REPORT OF A CASE

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AND

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About 120 cases of primary sarcoma of the spleen have so far been reported in the literature. The case described in this article is the only one in 6,400 autopsies performed at this hospital since 1884. There have been 7 cases of metastatic carcinoma in this series.

Smith and Rusk,¹ in 1923, made a survey of primary malignant tumors of the spleen, classifying the 104 cases which had been reported up to that time. They found a lack of uniformity in the terminology. As they brought out, there are three types of tissue in the spleen from which neoplasms may arise:

(1) the capsular and trabecular framework which may give origin to fibroma and fibrosarcoma, (2) the lymphoid elements from which may arise either a simple lymphoma (at times so-called lymphadenoma) or a malignant lymphoblastoma, that is, the so-called lymphosarcoma, and (3) angiomas and cavernous angiomas, together with the malignant counterpart, the endothelioma, arising from the vascular or sinus endothelium.

The tumors usually grow rapidly, and the diagnosis is usually made at necropsy.

Smith and Rusk decided that lymphosarcoma is probably the most usual type, although endothelioma is not uncommon. They reported 2 cases in which a tumor arose from the lining cells of the splenic sinuses. The cells assumed a definite alveolar arrangement for the most part. In some areas they were closely packed together with only fine connective tissue fibrils between them, as shown by Mallory's connective tissue stain. The cells had a fairly definite outline, spindle-shaped or polygonal, with clear, oval or rounded nuclei, the smallest being twice the diameter of lymphoid cells. Of the cases reported since that survey, we review briefly only those in which the microscopic picture was somewhat similar.

From the Blackburn Laboratory, Saint Elizabeths Hospital.

1. Smith, C. E., and Rusk, G. V. Arch. Surg. 7:371, 1923.

Taylor² described a spleen with a few atrophic malpighian bodies and an increase in the endothelial structures. The blood channels were large, poorly formed and lined by cells which were larger and more deeply stained than those normally present. There were many hyperchromatic cells with several closely packed nuclei or with one large vesicular nucleus. Mitotic figures were numerous. Taylor designated the endothelium of the splenic pulp as the origin of this tumor.

In Howard's case the clinical picture was that of a pernicious anemia. The spleen weighed 1,800 Gm., and there was metastasis only to the liver. The tumor cell was a reticulum cell with a round or ovoid vesicular nucleus and fairly abundant cytoplasm. There were occasional giant cells and many mitotic figures. Among these cells were varying numbers of lymphocytes, and a delicate reticulum was present throughout.

Wright and Stevenson⁴ described a sarcoma of the spleen composed of large cells and showing an alveolar arrangement. The main constituent of the tumor was a round cell with a fairly large nucleus, probably of mesoblastic origin. Cells of that type filled the meshes of a fine reticular stroma and produced a somewhat alveolar structure.

Paine⁵ reported another malignant neoplasm of the spleen which he called hemendothelioma. The spleen was enlarged and nodular. Some of the nodules were white and firm and were necrosed in the center. The nodules were separated by coarse and fine strands of fibrous tissue which from the arrangement beneath the capsule appeared to grow from the original splenic trabeculae. The normal pattern of the spleen was masked. Macroscopically, no malpighian corpuscles were identified. There were metastases to the liver and the bone marrow. The cells varied from a spindle-shaped form with a deeply stained nucleus to a nearly circular form with a vesicular nucleus. They resembled endothelial cells. Some of the cells showed phagocytic properties, and their tendency to form tubules indicated their endothelial origin. Intracellular fibrils were not demonstrated.

Caldwell⁶ summarized the observations in his case as follows: There was a primary malignant neoplasm with extensive metastases to the lymph nodes, lungs and subcutaneous tissues, together with numerous peritoneal implantations. The tumor was of the large round cell type with a tendency toward alveolar arrangement but without any definite angioplastic structure. Delicate angiophilic fibrils seemed to

2 Taylor, A. L. *Bristol Med-Chir J* **46** 121, 1929.

3 Howard, T. *J. Lab. & Clin. Med.* **14** 1157, 1929.

4 Wright, T. H., and Stevenson, E. M. K. *Glasgow M. J.* **114** 1, 1930.

5 Paine, C. G. *J. Path. & Bact.* **34** 139, 1931.

6 Caldwell, G. T. *South M. J.* **26** 120, 1933.

be formed by the tumor cells in the more differentiated portions of the tumor, but they were nearly completely lacking in the more cellular areas. The tumor was considered to be a primary reticulo-endothelioma of the spleen.

REPORT OF CASE

A well developed and well nourished white man of 42 years was admitted to the hospital on July 15, 1930, suffering from dementia paralytica. Previous to his admission he had received treatment with arsphenamine, mercury and malaria. The malaria terminated spontaneously after twelve chills. Serologic and neurologic findings confirmed the diagnosis of dementia paralytica. The patient showed complete mutism.

The clinical course was uneventful for nearly three years, then the inguinal lymph nodes began to enlarge. Abdominal palpation showed a large, hard, nodular mass at the crest of the left ilium. The roentgenologist's report read: "There is a disintegration of the wing of the left ilium. There is no evidence of a bony growth such as one would expect if the process were carcinomatous. It is possible that the condition is sarcoma." Further studies revealed numerous metastatic growths, but the location of the primary lesion could not be ascertained.

A biopsy specimen of an inguinal lymph node was reported on as follows: "The lymphoid tissue is practically completely destroyed and infiltrated by a new growth consisting of rather large polyhedral and spindle-shaped cells with large vesicular nuclei. There is a moderate amount of connective tissue stroma, rather slight on the whole, and small immature blood vessels. Occasional giant cell formation is observed. Many of the nuclei are large, undergoing mitotic division. The cells on the whole appear more of epithelial type although rather undifferentiated. In addition to the main cells there are numerous small rounded bodies, often slightly oval, staining intensely by hematoxylin and bearing no definite relationship to the cell, although they are occasionally found in the cell and even in the nucleus. They do not seem to indicate degeneration because there is not much necrosis present." The diagnosis was metastatic carcinoma.

Histologic examination of the tumor of the bone showed the following characteristics: "The appearance of the growth varies greatly from place to place. A section taken through the soft tissues shows an invasive growth made up of cells irregular in size and shape, with very little stroma. The cells have large nuclei and a tendency toward a fusiform or rounded cytoplasm. Nucleoli are present in some of the nuclei, and occasionally multiple nuclei are found in the same cell. There is invasion of the muscular tissue adjacent to the bone, infiltration between the separate fasciculi and degeneration of the muscular tissue. One of the sections from the bone shows rather normal-appearing red marrow, another shows a markedly increased number of small rounded oval cells and scattered large cells, considerably resembling those of the adjacent growth." The pathologist offered the following comments: "In comparison with the lymph node previously submitted, the tumor is apparently of the same nature, and is probably of epithelial origin. From the undifferentiated character of the cells, I should be inclined to regard it as highly malignant. I should hesitate to suggest the point of origin of this tumor, but the cells resemble those which might be derived from the lung or from the kidney." The histologic diagnosis was metastatic carcinoma.

At the time of this biopsy the patient's blood showed 74 per cent hemoglobin and an erythrocyte count of 4,010,000 cells per cubic millimeter. Two weeks later the hemoglobin content dropped to 45 per cent and the number of red blood cor-

puscles to 2,430,000. One month later the hemoglobin content was 44 per cent, and the red cells numbered 2,310,000. The white blood cell count remained normal throughout. The blood chemistry and electrocardiograms were not unusual. The basal metabolic rate was plus 20.2.

Considerable edema of the lower extremities appeared in the later stages. The patient showed progressive mental and physical deterioration. He died four months after the appearance of the first sign of neoplastic disease.

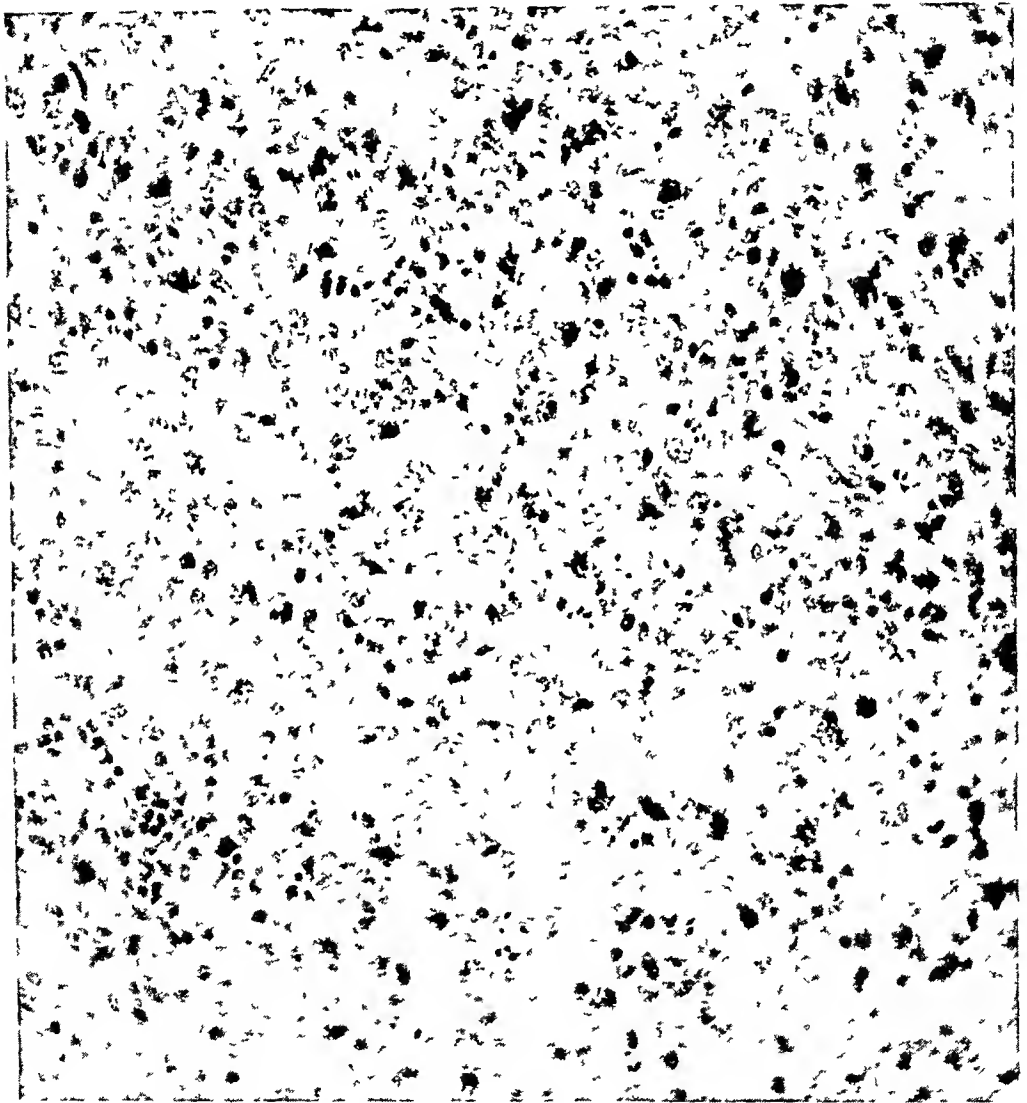


Fig 1—Section of the lymph node removed for biopsy. Hematoxylin and eosin stain, high power magnification.

A summary of the autopsy observations (made by Dr. Walter Freeman) follows. The body was poorly nourished. There was a large node in the left axilla. Two ribs showed tumor formation and pathologic fracture. There was induration of the left iliac bone. The parietal and visceral peritoneum showed numerous flat grayish plaques and was considerably thickened. The liver was

enlarged. Scattered areas of metastasis were found in the epicardium. Small tumor nodules were found in both lungs. These tumors were embedded in the tissue rather than compressing it. There were several indurated ulcers in the ileum. Metastatic growths were found in the serosa of the appendix and in the cecum, and a large nodule lay between the bladder and the rectum. The liver contained only one nodule, 2 cm in diameter. There was surprising preservation of the hepatic architecture in this nodule, and no obvious compression of the surrounding hepatic

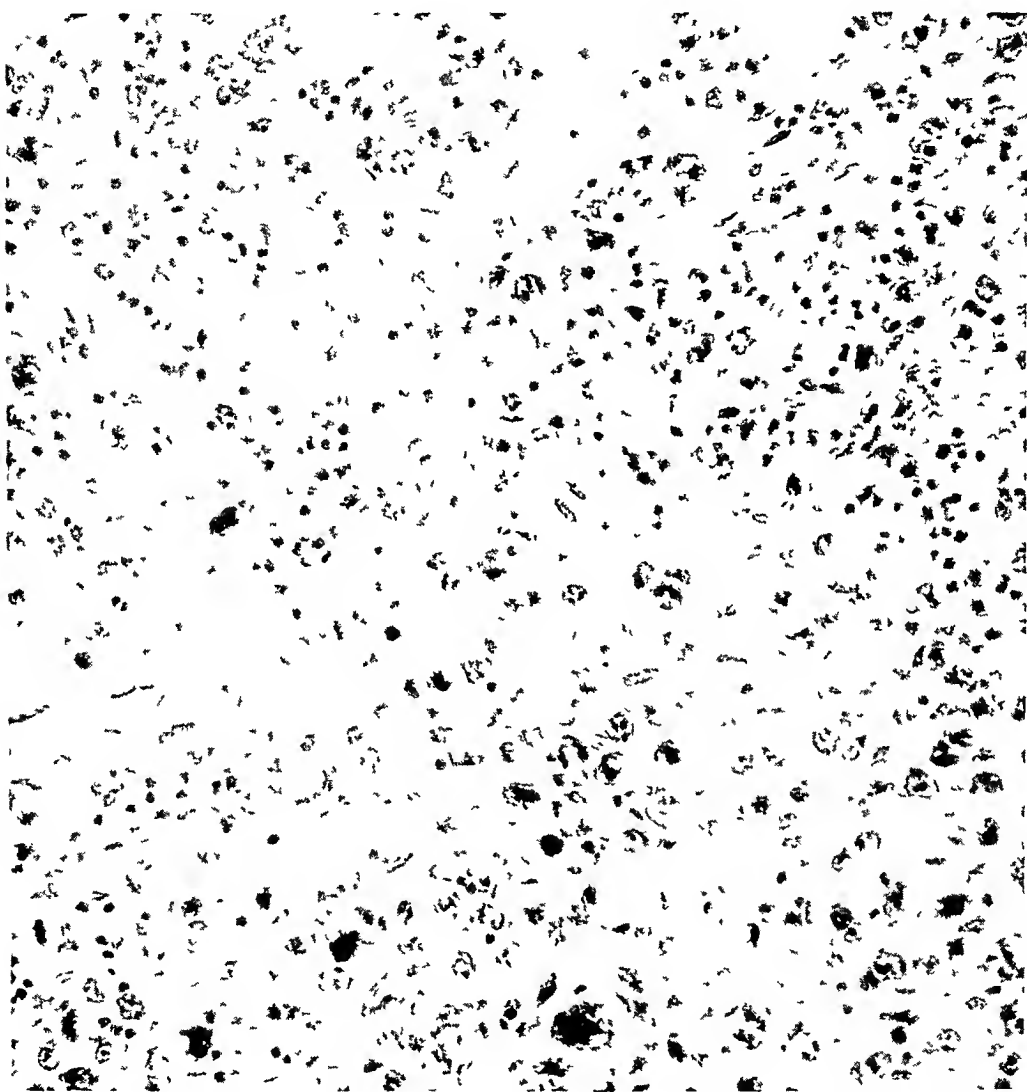


Fig. 2—Reticulo-endothelioma of the spleen. Hematoxylin and eosin stain, high power magnification.

tissue, as would be usual in the ordinary metastatic carcinoma. Metastatic nodules showing similar characteristics were also found in the pancreas.

The spleen weighed 695 Gm. It was dark, fairly firm and nodular. On incision it was divided up into a number of nodules, the largest being about 5 cm in diameter. Most of these were dark red, showing much of the color of

splenic tissue, except that in the center of some of them there was a stellate area of whitish color, which was slightly firmer than the surrounding dark splenic-appearing tissue but showed no tendency to necrosis. There was a little normal splenic tissue between the various lobules.

Confluent enlarged nodes were found along the abdominal aorta, around the left kidney, along the iliac and pancreatic vessels and in the left axilla. There



Fig 3—Reticulo-endothelioma of the spleen, showing argyrophilic reticulum Perdrau stain, high power magnification

were several nodules in the substance of each kidney. The bladder showed evidence of metastasis. The prostate and testes were normal. The endocrine system was normal, except for the right adrenal gland, in which metastasis was noted.

There was practically complete destruction of the sixth and seventh ribs in the axillary portion by the tumor involvement. A growth protruded from the

eighth and ninth ribs into the thorax and was adherent to the lung. A large mass appeared in the left ileum, which projected into the pelvis and disrupted the bone. There was also rather massive infiltration of the lumbar vertebrae. There were a few swellings beneath the pericranium which did not appear to invade the bone.

The brain apparently was normal.

The diagnosis at the time of autopsy was that there were possible sources of primary growth around the pelvis of the left kidney or the pancreas, with metastasis to the epicardium, lung, spleen, lymph nodes, peritoneum, liver, bladder, adrenal glands, ribs, vertebrae and ilium. However, it was mentioned that "the only other growth having definite appearances of primary carcinoma is in the spleen, where the foci are multiple and the character of the tissue entirely different from that seen in the other organs. The extent of the carcinomatous invasion of the bony system and of the peritoneal surface is quite unusual in this case, but the very great involvement here and the slight involvement in the thoracic cavity are a cause for comment."

Microscopic examination shortly after autopsy confirmed the first impression that the cell type was carcinoma originating in the region of the left kidney. However, it was mentioned that "the metastases are quite generally characterized by their infiltrative tendency, thus reminding one of lymphoid growths rather than carcinoma."

Detailed microscopic examination gave the following results. One section of the spleen included a portion of the tumor nodule with the whitish center, as described at autopsy, and also some compressed adjacent splenic tissue. The pallor of the center was due to the absence of blood and the scarcity of blood spaces in that area. There was a fairly definite fibrous capsule separating the neoplasm from the adjacent splenic pulp. This strip was not continuous and did not act as a barrier, as tumor cells were seen in the wall of a vessel which lies in this strip of connective tissue, also scattered in the pulp on the other side. A few trabeculae were seen in the sarcomatous area near the capsule.

In another section of this organ no trace of normal architecture was preserved. Except for the capsule, the splenic pulp had been entirely replaced with undifferentiated neoplastic cells. These cells were large, often attaining a size of 30 microns or more, with single or multiple vesicular nuclei and one or two prominent nucleoli. The rim of clear cytoplasm was usually narrow and assumed various shapes. Some of the cells were spindle-shaped with a long narrow nucleus, others were round, oval or polyhedral. Giant cells were fairly numerous. Mitotic figures were frequently seen. Small dark blue granules were often gathered in small groups enclosed in a cell body, but without nuclear membrane. Since the cells in which they appeared did not possess other visible nuclei, it was conjectured that they represented nuclear material or fragments of chromatin due to karyorrhexis. Sometimes similar-appearing granules were free in the tissue.

The few remaining malpighian corpuscles were small. Lymphocytes were scattered throughout the section, as well as a moderate number of polymorphonuclear leukocytes. Trabeculae were not recognized. The capsule was intact. Red blood cells were seen everywhere, but the walls of the vessels were thin and indistinct.

With the Perdrau stain there was seen to be an extremely dense anastomosing stroma of argyrophilic fibers everywhere. The vessels were now easily identified. The contrast with a normal spleen stained by this method was striking. In the normal section, connective tissue fibers were present only in the trabeculae and walls of sinuses. With Mallory's aniline blue connective tissue stain the picture

was about equally striking. Almost every individual cell appeared to be surrounded by a network of delicate and coarse fibrous strands.

The tumor cells were entirely similar in every metastasis. Studies were made of the metastases which occurred in the epicardium, lungs, kidneys, liver, pancreas, intestine, adrenal glands, omentum and ilium. A peculiar characteristic of this case is the intermingling of tumor cells and parenchyma without compression of the latter. The individual structure of the organ could be discerned underlying the neoplasm, except in the spleen, where the characteristic structure had been completely lost in the tumor. From our study of the case we concluded that the primary focus of the growth could not have been the kidney or the pancreas. From our microscopic study it appears that the 'stellate areas of whitish color' described at autopsy are centers of neoplastic growth. There is no doubt from the type of cells and their arrangement that we are dealing with a sarcoma instead of a carcinoma. The formation of an argyrophilic reticulum is proof that we are dealing with a mesodermal growth.

We classify it, therefore, as a primary sarcoma of the spleen originating in the reticulo-endothelial cells.

COMMENT

The lack of uniformity in the classification of splenic tumors has repeatedly been stressed. Ewing⁷ divided them into three groups: spindle cell sarcoma, endothelial sarcoma and lymphosarcoma. He expressed the belief that those arising in the reticulo-endothelial system are of most frequent occurrence. His description follows:

As a rule, it produces multiple nodules in a greatly enlarged organ, and many of these nodules may fuse into larger diffuse masses. Metastases are commonly present. The tumor is, therefore, quite malignant.

The structure consists of large cells with single or multiple vesicular nuclei and pale cytoplasm. They are round or elongated or polyhedral and giant-cells may form. The arrangement may be diffuse or alveolar.

Ewing did not mention the argyrophilic reticulum, which is a constant feature of tumors of reticulo-endothelial origin. The details of our observation show a great similarity to Ewing's description of endothelial sarcoma. We suggest, however, that all neoplasms of this nature be definitely classified as reticulo-endothelial sarcoma. This will avoid confusion with the angioplastic tumors arising in the endothelial lining of the sinuses.

⁷ Ewing, J. Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1931, p. 122.

GENERALIZED PRIMARY ANGIOSARCOMATOSIS OF THE LYMPH NODES

PERRY J MELNICK, M D

DECATUR, III

The following report concerns a unique case of generalized primary angiosarcomatosis of the lymph nodes. Only two cases resembling this one have been reported. Baumgarten¹ in 1916 described a case of generalized primary spindle cell sarcoma of the lymph nodes. Kaufmann² mentions a case of generalized primary myxosarcoma of the lymph nodes which he observed. A doubtful case reported by Spieler³ seems to be that of a reticulum cell lymphosarcoma (retiothel sarcoma) of the lymph nodes and intestine.

The familiar lymphosarcomas are by far the most frequent of the primary tumors of the lymph nodes. The so-called endotheliomas of lymph nodes are undoubtedly retiothel sarcomas, their histogenesis being from the sinus endothelium. Other primary sarcomas of the lymph nodes are rare. Kaufmann mentioned cases of fibrosarcoma, spindle cell sarcoma, angiosarcoma and round cell sarcoma. In these cases one lymph node or one group of lymph nodes was involved.

Our case is very curious and unique in that almost every lymph node in the entire body appears to be the site of apparently primary angiosarcoma. Baumgarten's case was that of a 58 year old man in whom at autopsy all the lymph nodes in the entire body were enlarged up to the size of hen's eggs and were the site of primary spindle cell sarcoma. No trace of tumor was found anywhere else. Kaufmann's case was of a 32 year old man with generalized lymph node enlargement of fifteen years' duration, in whom the lymph nodes were the site of myxosarcoma. A case ascribed by Spieler to this group turns out to be one of reticulum cell lymphosarcoma (retiothel sarcoma) of the lymph nodes and lymphatic tissue of the intestine. In his detailed description of the case the reticulum cells are fully identified even to the intracellular argentophil reticulum fibrils, but at the time of his report (1918) retiothel sarcoma had not yet been clearly recognized as one of the three forms of lymphosarcoma.

From the Department of Pathology Cook County Hospital Dr R H Jaffe director

1 Baumgarten P. Berl klin Wchnschr **52** 1201, 1915

2 Kaufmann, E. Pathology for Students and Practitioners, translated by S P Reimann Philadelphia, P Blakiston's Son & Co 1929 vol 1 p 282

3 Spieler F. Zur Lehre des generalisierten Sarcoms der Lymphdrusen und des Darmes Inaug Dissert Basel 1918

REPORT OF A CASE

The patient, a 64 year old Italian laborer, is alive and well. He entered the Cook County Hospital on Oct. 6, 1933. About a year previously he first noticed lumps in the neck, both axillary regions and both inguinal regions. The lumps grew slowly and never caused him any trouble. Four months before entrance he became short of breath and began to have swelling of the ankles. He had rheumatism twenty-four years before entrance. There was nothing else of significance in the past history, and the family history was negative.

He appeared well developed and well nourished, comfortable and not acutely ill. The pulse, temperature and respirations were normal. The blood pressure was 100 systolic and 55 diastolic. Examination of the head showed no abnormality. In the neck every group of lymph nodes on both sides was enlarged. The size of the nodes ranged to that of a cherry, and they were firm, discrete and movable. In the chest crackling rales were heard in both lower lobes but there were no other abnormal findings. The heart, on percussion, appeared slightly enlarged to the left, a systolic murmur was heard over the apex, transmitted to the axilla. In both axillary regions the lymph nodes were enlarged to walnut size. They were discrete and firm. In addition, every other group of lymph nodes in this region was enlarged, namely subpectoral and interpectoral nodes. These could not always be seen but could be felt as pea-sized movable nodes. The abdomen was soft and not tender. The liver was palpable 2 fingerbreadths below the costal margin. The spleen and kidneys were not palpable, no abnormal masses were felt. In the inguinal regions the lymph nodes were enlarged to walnut size, discrete and firm. In the upper extremities the epitrochlear lymph nodes were enlarged to cherry size, and several smaller nodes were palpable, forming a chain along the medial aspects of both arms. The lower extremities had slight pitting edema about the ankles.

Leukemia, lymphosarcoma or Hodgkin's disease were the possibilities that came to mind. The cardiac findings were interpreted as a rheumatic mitral lesion with myocardial decompensation. Examination of blood films revealed no abnormal cells. There was a moderate anemia, the hemoglobin content was 66 per cent, the red blood cell count, 3,470,000 the white blood cell count, 10,200. Stained films revealed no abnormal blood cells. The urine was normal, the blood chemistry was normal and the Kahn reaction of the blood negative. An x-ray film of the chest revealed only slight increase in the hilus markings. X-ray films of the gastrointestinal tract and of the skeleton revealed nothing abnormal.

A biopsy of a cervical lymph node disclosed what was apparently an angiosarcoma associated with a lymph node. A biopsy of another cervical lymph node revealed an exactly similar picture. This unusual finding stimulated much interest in the case. Still another biopsy was made, and this time two large cherry-sized lymph nodes were carefully dissected out from the right epitrochlear region and immediately fixed in Zenker's fluid containing dilute formaldehyde solution. The patient made an uneventful recovery from the biopsies and with digitalis therapy for his heart condition improved so markedly that he insisted on going home. He is at the time of writing alive and well, thirty months after the first appearance of the enlargement of the lymph nodes. A recent examination revealed nothing additional of importance. The nodes have increased only very slightly in size in the past fifteen months, and are giving the patient no trouble (fig. 1).

Microscopic Examination—Sections of the lymph nodes fixed in Zenker's fluid were stained with the hemalum eosin, Van Gieson, Mallory phosphotungstic acid

hematoxylin, elastic tissue and iron stains. The histologic appearance of all the excised lymph nodes is the same. The greater part of the center of each node is composed of tumor tissue (fig 2). In the periphery of the node a rim of intact lymphatic tissue is seen which is slightly compressed but otherwise shows no changes. The centrally located tumor tissue is separated from this peripheral rim by a narrow band of dense connective tissue. The tumor tissue making up the center of the lymph node is composed uniformly of narrow vascular spaces lined by flat endothelium. These spaces are about the diameter of small capillaries and contain red blood cells. Between the vascular spaces is a delicate stroma



Fig 1—Recent photograph of the patient showing enlarged axillary, pectoral, cervical and epitrochlear lymph nodes. The biopsy scar in the right epitrochlear region is visible.

This stroma is composed of fusiform and spindle-shaped cells with elongated dark-staining nuclei. Scattered throughout are a few delicate collagen fibrils. The stroma shows intimate relations to the vascular spaces. In many places these vascular spaces can be seen in the process of formation as young capillary twigs budding from the fusiform cells. There is little anaplasia, and only occasional mitotic figures can be seen. The vasoformative character of the undifferentiated stroma composed of fusiform cells and its relation to the vascular spaces are clearly seen in all the nodes examined (fig 3).

COMMENT

Several interesting questions present themselves. First is the possibility of the condition being one of multiple metastases. The absence of a primary source and the absence of visceral and skeletal metastases



Fig 2—Very low power view of a lymph node showing centrally located tumor tissue and a peripheral rim of lymphatic tissue separated from the tumor by the latter's narrow connective tissue capsule

speak against this. Such extensive multiple metastases to lymph nodes would be exceedingly rare. Of course the possibility still exists, because after all only biopsy material was studied. But it is unlikely since exhaustive clinical investigation of the patient failed to reveal a primary tumor.

Second is the relationship of the tumor to the lymph nodes themselves. There seems to be no relation to the lymphatic tissue proper. The tumor is separated from the lymphatic tissue by a narrow band of collagen fibers and fibrocytes. Some other structure in the lymph node seems to have been the point of origin. Apparently vascular anlagen in



Fig 3—High power view of the tumor tissue (hemalum and eosin). Capillary spaces are seen budding from the fusiform cells of the stroma.

either the capsule, the trabeculae or the hilus, or the preexisting blood vessels of the lymph node, may account for the histogenesis.

Third, the possible relationship to the reticulo-endothelial apparatus of the lymph nodes must be considered. There seems to be no relationship. The tumor has distinctly the character of angiosarcoma. The

fusiform stroma cells are of differentiated angioblastic type and have no resemblance to or relationship with, the reticulum cells. The vascular spaces are lined by ordinary vascular endothelium, which, as is well known, has no relationship to reticulo-endothelium.

The question of the degree of malignancy of the tumor is interesting. Since there is so little cellular anaplasia, since the patient's condition is still good, and since the lymph nodes have grown only very slowly in the past thirty-two months the degree of malignancy seems to be low. One is reminded of Kaufmann's case of fifteen years' duration, and of Kaposi's multiple angiosarcomatosis of the skin in which durations up to twenty-five years have been reported.

The impression in this case is that the tumors have arisen from independent multiple angioblastomatous anlagen in the lymph nodes. Such multiple systemic angiomatosis is not at all rare. Osler's disease or multiple telangiectasia of the skin and internal organs, for instance, is well known. This hereditary condition has been traced in about one hundred families known to have the disease. It is characterized by the occurrence of multiple discrete hemangiomas in the skin and internal organs. Jaffe⁴ and Goldstein⁵ have made important studies of this condition. It illustrates the occurrence of multiple discrete angiomatous anlagen.

Of much greater resemblance to the present case, however, is Kaposi's multiple angiosarcomatosis of the skin. The histologic picture of this lesion is characteristic. In the early lesions oval to spindle-shaped cells appear and proliferate, and forms in transition from them up to well formed capillary-like vascular spaces are seen. The fusiform cells produce a vasoformative tissue from which the small capillary buds grow. Dorffel⁶ has made an extensive histologic study of sixteen cases of Kaposi's sarcomatosis and finds this histologic picture to be characteristic. In the late stages this picture is changed and obscured by hemorrhage and degenerative changes. Gans⁷ in his textbook gives the same characteristics. Hamdi and Resat⁸ found the same histologic picture in the early lesions in several cases. They also proved that the lesions are discrete and independent of each other by making serial sections through a number.

The striking similarity of the histologic picture in the present case to that of Kaposi's sarcomatosis is of more than passing interest. In

4 Jaffe, R. H. *Arch. Path.* **7** 44, 1929.

5 Goldstein, H. I. *Arch. Dermat. & Syph.* **26** 282, 1933.

6 Dorffel, J. *Arch. Dermat. & Syph.* **26** 608, 1932.

7 Gans, O. *Histologie der Hautkrankheiten*, Berlin, Julius Springer, 1928, vol. 2, p. 463.

8 Hamdi, H., and Resat, H. *Ann. d'anat. path.* **9** 593, 1932.

both, the tumor tissue is vasoformative, that is composed of undifferentiated fusiform mesenchymatous cells from which capillary buds grow. Orsos⁹ has made a histologic study of eleven cases of such capillary angioblastic tumors. They are quite different from angioendotheliomas, in the latter the differentiated endothelial cells lining the vascular space are the proliferating units.

SUMMARY

The case reported here is one of generalized primary angiosarcomatosis of the lymph nodes of thirty-two months' duration in a 64 year old white man. Biopsies of four different lymph nodes revealed the same picture, namely, that of an angioblastomatous tumor of low grade malignancy centrally located in the lymph nodes fairly well encapsulated and apparently independent of the lymphatic tissue or the reticulo-endothelial apparatus. The origin seems to be from multiple vasoformative anlagen in the lymph nodes. The relationship to other forms of multiple angiomatosis and to Kaposi's multiple angiosarcomatosis of the skin is discussed. Two similar cases of generalized primary sarcomatosis of the lymph nodes have previously been reported.

9 Orsos F. Beitr. z. path. Anat. u. z. allg. Path. **93** 121, 1934.

General Review

TRYPANOCIDAL ACTION OF NORMAL HUMAN SERUM

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The trypanocidal property of human serum has been known since 1902, when Laveran¹ discovered the phenomenon while studying mice experimentally infected with the trypanosome of nagana, *Trypanosoma Brucei*. In the years following this observation, the investigations in the field, which have taken numerous directions, have gradually indicated that this property of the serum is of considerable significance in immunology. Although a large number of papers have been presented on the subject, no general summary of the trypanocidal phenomenon has appeared in recent years covering the various aspects which research on this interesting and striking characteristic of human serum has taken. It is to supply this need for the increasing number of workers in this and related fields that the present review is offered.

When studied with the purpose to explain the development of natural antibodies in general, the trypanocidal property of human serum takes on added significance. The average person probably at no time in his life is exposed to subclinical or latent infection or to specific immunization by other channels with any form of trypanosome. Yet, regularly, this antagonistic property develops in the blood of normal human beings soon after birth. The chance of heterologous immunization with some other agent which possesses antigenic moieties in common with the trypanosome remains, however, although as yet no such agent has been determined. It seems possible that the trypanocidal substance occurs in consonance with the normal physiologic maturation of the human body² and is not to be regarded as the product of a process of antibody formation. In the light of recent observations, it is perhaps not too much to hypothesize that analogous processes are responsible for the appearance in the serum of normal persons of antagonistic substances of a broad range, including those which destroy certain bacteria and certain filtrable viruses.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University

1 Laveran, A. *Compt. rend. Acad. d. sc.* **134** 735, 1902

2 Jungeblut, C. W., and Engle, E. T. *J. A. M. A.* **99** 2091, 1932

In reviewing the subject, I shall discuss the limitations and the general properties of the trypanocidal substance, its formation by the body, its mode of action on trypanosomes, its correlation with man's immunity against trypanosomes, its possible clinical significance and, finally, its relationship to certain other protective activities of the serum

LIMITATIONS OF THE TRYPANOCIDAL PROPERTY

Species of Trypanosomes Affected—Human serum manifests its activity not only on the trypanosome of nagana (*T. Brucei*) but also on the trypanosomes of surra (*T. Evansi*), mal de caderas (*T. equinum*) and dourine (*T. equiperdum*) as well as on other trypanosomes naturally pathogenic for animals³. Normally, these trypanosomes possess great virulence for mice and rats, and such animals generally die in from three to five days after the parasites have appeared in the blood stream⁴. When 1 cc of human serum is introduced into the infected animals, however, the parasites, which may have been present in tremendous numbers previously, disappear completely from the blood within a few hours. After an interval of from eight to twenty days, the trypanosomes return and, unless more human serum is introduced, promptly cause the death of the animal. Such removal of the parasites from the circulation can be accomplished repeatedly over a period of two or three months, but finally the effectiveness of the human serum is lost and the animal dies. Laveran⁵ noted that only very seldom was complete cure or sterilization possible through the administration of human serum. Nattan-Larrier and Noyer,⁶ likewise, frequently observed relapses after the injection of serum, and Adams⁷ failed to effect cure at any time, although the temporary removal of parasites from the blood was accomplished repeatedly. The use of human serum is more successful for the purpose of preventing than of treating infections with the pathogenic trypanosomes⁸. However, amounts as small as 0.1 cc of normal serum injected subcutaneously into a mouse of 20 Gm weight at the time of infection with *T. Brucei* often are capable of causing complete inhibition of the infection.

A very striking difference in the effect of human serum is seen in mice infected with the pathogenic trypanosomes of man, *T. gambiense*,

3 (a) Laveran, A. *Compt rend Acad d sc* **137** 15, 1903. (b) Thirouz, A., and d'Anfreville, L. *ibid* **147** 462, 1908. Laveran¹

4 Tahaferro, W. H. *Quart Rev Biol* **1** 246, 1926.

5 Laveran, A. *Compt rend Acad d sc* **138** 450, 1904.

6 Nattan-Larrier, L., and Noyer, B. *Compt rend Soc de biol* **104** 475, 1930.

7 Adams, P. *Ztschr f Immunitätsforsch u exper Therap* **58** 456, 1928.

8 (a) Goebel, O. *Ann Inst Pasteur* **21** 882, 1907. (b) Rosenthal, F., and Krueger, M. *Berl klin Wchnschr* **58** 382, 1921.

T rhodesiense and T Cruzi⁹ If used in the test soon after their isolation from human beings with trypanosomiasis, these parasites are insusceptible to the action of human serum Somewhat readily, however, T rhodesiense, in distinction from the other human forms, becomes susceptible to the effects of human serum after repeated passage through mice, rats or guinea-pigs¹⁰ Corson¹¹ has found that human serum is more effective in mouse infections with T rhodesiense if the parasites are first passed through sheep and goats Mesnil¹² reported that a strain of T gambiense also became susceptible to human serum after being passed through animals in his laboratory for nine years, but another strain kept even longer, twelve years by Laveran¹³ retained throughout this period its original serum resistance This selectivity in the action of human serum is considered by some to be of importance in connection with man's immunity to the animal pathogens and his susceptibility to the human species of trypanosomes More will be said on this point later

The common, nonpathogenic parasite of rats, *Trypanosoma Lewisi*, is also insusceptible to the trypanocidal effect of human serum This was noted by Laveran and Mesnil¹⁴ and recorded in one of their earliest papers I¹⁵ also have found T Lewisii infections in rats unaltered in any phase of their course¹⁶ by the administration of human serum The resistance of T Lewisii to human serum parallels its resistance to most of the chemicals which act so positively on the pathogenic trypanosomes

I have found recently that *Trypanosoma diemyleti*, the trypanosome of the red-spotted newt, *Triturus viridescens*, also is resistant to fresh human serum even when the newts are kept at a temperature of 37 C after the injection of the serum

Type of Serums Which Exhibit Trypanocidal Power—The trypanocidal power of serum is not widespread among the species of animals It is, indeed, almost exclusively a property of the serum of man The serums of some kinds of monkeys are active, however Large doses

9 (a) Laveran, A, and Nattan-Larrier, L Compt rend Acad d sc **154** 18, 1912 (b) Zeiss, H Arch f Schiff's- u Tropen-Hyg **24** 73, 1920 Laveran⁵

10 Mesnil, F, and Ringenbach, J (a) Compt rend Acad d sc **153** 1097, 1911, (b) *ibid* **155** 78, 1912, (c) Bull Soc path exot **7** 612, 1914 (d) Fairbairn, H Ann Trop Med **27** 251, 1933

11 Corson, J F J Trop Med **34** 81, 1931

12 Mesnil, F Compt rend Soc de biol **77** 564, 1914

13 Laveran, A Bull Soc path exot **8** 442, 1915

14 Laveran, A, and Mesnil, F Ann Inst Pasteur **18** 785, 1902

15 Culbertson, J T Ann Trop Med **28** 93, 1934

16 Taliaferro, W H J Exper Med **39** 171, 1924

of the serum of the baboon, which is naturally immune to infection with all species of trypanosomes,¹⁷ is effective even against the human forms, *T. gambiense* and *T. rhodesiense*, which are resistant to human serum.¹⁸ In its action on the animal pathogens (*T. Brucei*, *T. equiperdum*, etc.), on the other hand, baboon serum is less powerful than human serum.¹⁹ The serums of mangabeys and mandrills also exhibit limited activity against *T. Brucei*.²⁰ In contrast to these positive effects with the serums of certain Primates, no activity against any species of trypanosome has been observed with the serum of any of the common domestic or laboratory animals (horse, cow, sheep, goat, pig, rabbit, guinea-pig, rat, mouse and fowl).

Hosts in Which the Trypanocidal Effect of Human Serum Is Manifested—The earliest work on the trypanocidal activity of human serum was carried out in mice and rats. Goebel,^{8a} and more recently Peruzzi,¹⁹ employed guinea-pigs in some of their tests, but the results obtained have been somewhat less constant than those obtained with mice and rats. Larger animals have been employed but rarely, probably because of the proportionately greater volume of human serum required to demonstrate the trypanocidal effect.

PROPERTIES AND NATURE OF THE TRYPANOCIDAL SUBSTANCE

Authors are in general agreement concerning many of the basic properties of the trypanocidal substance in human serum, but observations on some other characters are in such conflict that conclusions are reached only with difficulty. It is established that the trypanocidal substance is found in the fluid and not in the formed part of the blood.²² Goebel^{8a} early associated the substance with the globulin fraction of the serum, and this has found confirmation with Rosenthal and Freund²³ and others. The substance is insoluble in ether.²⁴ It is thermolabile; heating at 64 C. for an hour destroys wholly the activity of the serum, and exposure at a temperature of 56 C. for this interval leads to its

17 Zschucke, J. Arch. f. Schiffs- u. Tropen-Hyg. **37** 194, 1933. Regendanz, P. *ibid.* **37** 195, 1933.

18 Laveran, A. Compt. rend. Acad. d. sc. **139** 177, 1904. Mesnil and Ringenbach.^{10a}

19 Peruzzi, M., in Final Report of League of Nations International Commission on Human Trypanosomiasis, Boston, World Peace Foundation, 1928.

20 Mesnil, F., and Leboeuf, A. Compt. rend. Soc. de biol. **69** 382, 1910.

22 (a) Citron, H. Ztschr. f. Immunitätsforsch. u. exper. Therap. **27** 369, 1918. (b) Rosenthal, F. *ibid.* **62** 464, 1929. (c) Pfannenstiel, W., and Scharlau, B. Centralbl. f. Bakt. (Abt. 1) **110** 84, 1929.

23 Rosenthal, F., and Freund, R. Ztschr. f. Immunitätsforsch. u. exper. Therap. **37** 48, 1923.

24 Jacoby, M. Ztschr. f. Immunitätsforsch. u. exper. Therap. **2** 689, 1909.

partial inactivation²⁵ The addition of fresh guinea-pig serum fails to restore the original trypanocidal potency of a serum rendered inactive by heat²⁶ When allowed to stand at room temperature, human serum gradually loses its trypanocidal power and in two or three months is totally devoid of activity^{3a} Even after a few days in the icebox a significant loss is noted, and after from two to four weeks marked reduction in the power is observed⁷ If, however, the serum is dried and the powder dissolved in a suitable fluid when needed, activity is retained more or less quantitatively for at least six months^{3a}

Relationship to the Serum Antibodies—Whether or not the trypanocidal substance is identical with the usual serum antibodies is not established Laveran and Mesnil¹¹ and Goebel^{8a} believed it unlike an opsonin, and others²⁷ have considered it different from any of the usual serum antibodies Laveran and Mesnil¹⁴ found that human serum failed to agglutinate trypanosomes whereas goat or sheep or especially pig serum—all of which are entirely without trypanocidal action—gave marked agglutination They also discovered that fowls, which are refractory to trypanosomes, yield, after long immunization with a suspension of trypanosomes, a serum devoid of trypanocidal property At the same time, however, these workers found that the serum of goats or sheep immunized against trypanosomes prevented infection in mice if mixed with the trypanosomes when these were injected, although if the immune serum was inoculated elsewhere in the animal at the time of infection no protection resulted On the other hand, Strong and I²⁸ observed that human serum loses its trypanocidal power after absorption with a mass of trypanosomes as well as with a mass of certain species of bacteria (e g, the typhoid bacillus and *Proteus*) Hence we concluded that the trypanocidal substance is a relatively nonspecific antibody

Relation to Alexin—It was postulated by A R D Adams²⁵ that "complement is the trypanocidal substance" because both properties were destroyed by heating, filtering or ammonifying a serum In support of this, it has been found that both serum properties are lost or reduced simultaneously in certain types of disease²⁹ Goebel,^{8a} however, has thought the trypanocidal substance distinct from complement or alexin since the trypanocidal power, once inactivated by heating, cannot

25 Adams, A R D Ann Trop Med 25 299, 1931 Adams⁷ Goebel^{8a} Laveran and Mesnil¹⁴

26 Adams⁷ Goebel^{8a}

27 Rosenthal, F, and Freund, R Ztschr f Hyg u Infektionskr 97 137, 1922 Adams⁷

28 Culbertson, J T, and Strong, P S Am J Hyg 21 1, 1935

29 (a) Lange Klin Wchnschr 1 1040, 1922 (b) Freund, R, and Gassmann ibid 8 233, 1929

be reactivated by the addition of fresh guinea-pig serum. Furthermore, it was pointed out that the trypanocidal action is a characteristic of the blood only of man and several kinds of apes whereas alexin is common to all forms³⁰. Handler³¹ has reported that no more than a crude parallelism exists between the rates of inactivation of the trypanocidal substance and that of alexin when fresh human serum is permitted to stand in the refrigerator. Strong and I²⁸ in studies in vitro have found that the trypanocidal substance is separable from all of the known components of alexin both by filtration through Berkefeld candles and by the specific inactivation of each of the components of alexin. Furthermore, we have succeeded in totally removing the trypanocidal substance from the serum by absorption at 0 C with typhoid bacilli without reducing significantly the potency of the alexin. We have concluded not only that the trypanocidal substance is distinct from alexin but that each of these serum properties can manifest its activity in the complete absence of the other.

Antigenic Property of the Trypanocidal Substance—The antigenicity of the trypanocidal substance has been a point of special interest. Laveran and Mesnil¹⁴ offered an experiment from which they concluded that no antitypanocidal antibody was formed as the result of repeatedly treating an animal with a trypanocidal serum. An infected rat was given seven injections of 2 cc of human serum at the rate of one every second day, beginning two days after infection with T Brucei. The trypanosomes were not affected by the last injection of the series, and the rat's blood was drawn. When 0.5 cc of this blood was mixed with 0.5 cc of human serum and this mixture injected into an infected rat, the trypanocidal action of the human serum was still demonstrable. Goebel,^{3a} on the other hand, found that human serum after being mixed with rabbit antihuman serum lost part of its curative power and was no longer protective. Rosenthal and Freund²³ explained the gradual loss of trypanocidal power after repeated injections of human serum, not on the basis of antibody formation, but by an "exhaustion" mechanism, which will be discussed later. Recently, Handler³¹ presented what is perhaps the most satisfactory evidence on the problem of antigenicity of the trypanocidal substance of serum. He concluded that the trypanocidal substance has the capacity to produce an equivalent precipitating antibody separate from those formed against other proteins of the serum, and has been able completely to inactivate in vitro the trypanocidal substance in human serum by precipitation with an optimum amount of a specific immune serum from the rabbit. Neither the supernatant fluid over the precipitate nor the precipitated

30 Rosenthal, F. Klin Wchnschr 3 1657, 1924. Rosenthal and Freund²⁷

31 Handler, B. J. Am J Hvg 21 18, 1935

substance manifested trypanocidal activity. Handler³¹ considered the trypanocidal substance antigenically distinct from other fractions of the human serum because an immune serum prepared against a human serum in which the trypanocidal substance has been inactivated by heat was incapable of neutralizing the trypanocidal substance of a fresh human serum. The same author demonstrated similar antagonistic effects *in vivo* by treating infected mice with human serum and, separately, with rabbit antihuman serum. It seems probable from this that the trypanocidal substance of the human serum is antigenic and gives rise to a specific neutralizing antibody on injection into animals. The inability of some earlier workers to demonstrate the inhibitory effect of antihuman serum on the trypanocidal power of normal human serum probably lies in their failure to observe necessary quantitative relationships between the antigen and the antibody concerned.

Additional Properties—A number of other properties of the trypanocidal substance should be mentioned. Adams⁷ found the trypanocidal action more marked when blood was permitted to clot at 37 C. or at room temperature than when placed at 0 C. With him, serums that gave a positive Wassermann reaction exhibited a greater trypanocidal power than those that gave a negative Wassermann reaction. Laveran and Mesnil¹⁴ believed that the rapidity of the removal of parasites depended on the number present, but noted that frequently the injection of 0.5 cc. of serum was more effective than the administration of twice this quantity. Johnson,³² however, has been unable to demonstrate any such zone phenomenon in connection with the action of human serum. The trypanocidal substance passes readily through Berkefeld candles,²⁸ although it is partly lost by ultrafiltration,³³ the latter property again differentiating it from alexin. The trypanocidal substance probably does not pass the placenta.³⁴

FORMATION OF THE TRYPANOCIDAL SUBSTANCE IN THE HUMAN BODY

One of the early observations of Laveran and Mesnil¹⁴ was that while the serum of a human being exhibited marked trypanocidal power, the pleural fluid, ascitic fluid and possibly even the blood plasma showed much less activity. It was thought, therefore, that the trypanocidal substance emanated from the leukocytes and was freed or separated from them during blood coagulation. Body fluids which possessed few leukocytes would, accordingly, be potentially poor in trypanocidal power.

³² Johnson, T. L. *Am. J. Hyg.* **9**: 260 and 283, 1929.

³³ Nattan-Larrier, L., and Nover, B. *Compt. rend. Soc. de biol.* **105**: 630, 1930.

³⁴ Nattan-Larrier, L., and Lepine, P. *Compt. rend. Soc. de biol.* **97**: 1470, 1927.

Salmon,³⁵ however, injected the leukocytes from two kinds of pus as well as an extract of a lymphatic gland without eliciting the trypanocidal action. When he tried serous exudates, he found, in contrast to the results of Laveran and Mesnil,¹⁴ marked trypanocidal effects. Salmon³⁵ decided, therefore, that the substance was a property of the blood fluid and not of any of the blood cells. This has been confirmed by Rosenthal^{22b} and Pfannenstiel and Schailau.^{22c} Salmon³⁵ and Regendanz³⁶ have found cerebrospinal fluid inactive. The urine has no trypanocidal power.³⁷ Rosenthal^{22b} also has found emulsions of various organs inactive.

The Liver as the Site of Formation of the Trypanocidal Substance—Several investigators have noted that in the serums of patients suffering from certain types of liver disorders the trypanocidal activity is sharply reduced.³⁸ Rosenthal³⁰ and Rosenthal and Nossen³⁹ concluded that the trypanocidal substance found in human serum is a product of the normally functioning healthy liver and is decreased in amount as certain pathologic conditions disturb this normal function. A large amount of important work in connection with the diagnosis of liver disorders has resulted. As the problem now stands, there is general agreement with Rosenthal that the trypanocidal substance is formed by the healthy liver.

Appearance of the Trypanocidal Property in Infants—It is recognized that the serum of very young infants is less active in trypanocidal power than is that of adults.⁴⁰ Neumark and Pogorschelsky^{40b} believed the trypanocidal substance was absent in children up to the age of 3 weeks and gradually increased during the first three months of life. Levy,^{40c} however, has found the substance in a large percentage of the serums of children 3 weeks of age, some of whom unfortunately could not be considered to be in normal good health, and Nattan-Larrier and Lépine³⁴ have detected it even in the blood of a new-born infant. Nattan-Larrier and Lépine³⁴ believed that the activity of the serum of very young children was not due to passage of the trypanocidal substance through the placenta but to the precocious development of this substance in the young child. Rosenthal and Kleeman^{40a} and others⁴¹

35 Salmon, P. *Bull Soc path exot* **3** 726, 1910.

36 Regendanz, P. *Zentralbl f Bakt (Abt 1)* **120** 89, 1931.

37 Rosenthal^{22b} Salmon³⁵

38 (a) Ehrlich, P. *Berl klin Wchnschr* **44** 233, 1907. (b) Platau, L. *Ztschr f Hyg u Infektionskr* **81** 401, 1916. Rosenthal and Krueger^{8b}

39 Rosenthal, F., and Nossen, H. *Berl klin Wchnschr* **58** 1093, 1921.

40 (a) Rosenthal, F., and Kleeman, E. *Berl klin Wchnschr* **52** 75, 1915. (b) Neumark, E., and Pogorschelsky, H. *Klin Wchnschr* **4** 1725, 1925. (c) Levy, S. *Jahrb f Kinderh* **120** 325, 1928. Laveran^{3a} Nattan-Larrier and Lépine³⁴

41 Eufinger, H., Rothmundt, M., and Wiesbader, H. *Monatschr f Geburtsh u Gynak* **93** 249, 1933.

reported an increase in the trypanocidal power of the serum of pregnant women in the last part of the gestation period, and Neumann⁴² noted that the serum of pregnant women was powerfully trypanocidal directly after term. Rosenthal and Kleeman^{40a} obtained no evidence for the transmission of the substance from the mother to the child during nursing since the milk lacked trypanocidal activity.

The trypanocidal potency of the serum of children is subject to more marked fluctuation than is that of adults. This has been explained by assuming the building up of a store of the substance in the fluids and tissues as the child becomes older.⁴³ Rosenthal and Nossen³⁹ believe that the lack of the substance in very young children is due to the incomplete functioning of the organs of the young child.

A considerable variation occurs in the trypanocidal potency of the normal serums of healthy adult persons.⁴⁴ Whether there is a relationship between the concentration of this substance and the human blood groups is not as yet indicated. This point may deserve investigation since somewhat greater resistance to certain diseases has been reported in persons of certain blood groups.⁴⁵

MODE OF ACTION OF THE TRYPANOCIDAL SUBSTANCE ON THE PARASITE

The mode of action of the trypanocidal substance on trypanosomes is not well understood. Some have felt that it behaves as an antibody, possibly requiring the presence of alexin for the manifestation of its activity. Others believe the trypanocidal substance acts essentially as a chemotherapeutic agent, perhaps complemented by some element elaborated in the body of the animal in which the trypanocidal action takes place. The development by Yorke and his co-workers⁴⁶ of a method for demonstrating the trypanocidal effect of serum wholly in vitro has greatly facilitated study of the problem and has made possible the correction of certain faults in theory which previously had been accepted.

The Trypanocidal Substance as an Antibody—There is no reason to believe that the trypanocidal action is opsonic.⁴⁷ It was early recognized that phagocytosis of the living parasites was not stimulated as a result of administering human serum although leukocytes were observed

42 Neumann, R. Ztschr f Hyg u Infektionskr **69** 109, 1911

43 Grunmandl, S., and Leichtentritt, B. Jahrb f Kinderh **106** 203, 1924
Rosenthal and Nossen³⁹

44 Laveran and Mesnil¹⁴ Plateau 38b

45 Lattes, L. Individuality of the Blood in Biology and in Clinical and Forensic Medicine, New York, Oxford University Press, 1932

46 Yorke, W., Adams, A. R. D., and Murgatroyd, F. Ann Trop Med **23** 501, 1929

47 Laveran¹ Goebel^{8a} Laveran and Mesnil¹⁴

to engulf dead trypanosomes in the blood stream of a serum-treated mouse Rosenthal and Spitzer⁴⁸ found the trypanocidal power not reduced in aleukocytic animals—that is, animals treated with thorium-x to reduce the number of leukocytes There seems at present no reason to believe that either leukocytes or opsonins are of importance in the trypanocidal activity of human serum

Considerable debate has occurred as to whether or not the mechanism of action of the trypanocidal substance is the same as that of alexin and sensitizer Goebel^{8a} insisted that serum acted neither preventively nor curatively by such a mechanism and believed that the loss of activity by heating, aging and treating the serum with alkali—which appeared to favor the function of such a mechanism—could be explained otherwise than by inactivation of alexin Goebel obtained no fixation in vitro by trypanosomes of any substance to which the serum owed its activity With him, human serum digested at 37 C with trypanosomes retained its preventive and curative properties, and the exposed parasites likewise retained their infectivity Because of his failure to effect fixation of the trypanocidal substance to either trypanosomes or yeast cells (the latter of which had been shown by von Dungern⁴⁹ to fix alexin) and because of his inability to obtain reactivation with fresh guinea-pig serum, Goebel^{8a} concluded that the trypanocidal activity was due to something in the serum other than alexin Recently, Rosenthal,^{22b} in agreement with Goebel,^{8a} found no fixation of alexin in vitro and considered the trypanocidal action unlike that of any of the usual serum antibodies, a point to which Adams⁷ also agreed Apparently the reaction between normal human serum and susceptible trypanosomes is unlike that occurring between an extract of trypanosomes and the serum of a recovered or an infected animal, because fixation occurs in the latter case Indeed, the test for alexin fixation has had considerable use in the diagnosis of trypanosome infection among animals⁵⁰ as well as in the classification of the trypanosomes⁵¹ Furthermore, complement-fixing antibodies of rather limited specificity can be demonstrated in the serum of guinea-pigs either after injection of dead trypanosomes or after infection with the parasites

The Trypanocidal Substance as a Chemotherapeutic Agent—The Exhaustion Phenomenon—Rosenthal⁵² recently has concluded that the

48 Rosenthal, F, and Spitzer, F Ztschr f Immunitätsforsch u exper Therap **40** 529, 1924

49 von Dungern, F Munchen med Wchnschr **47** 677, 1900

50 Levaditi, C, and Mutermilch, S Ztschr f Immunitätsforsch u exper Therap **2** 702, 1909 Schoening, H W J Infect Dis **34** 608, 1924 Landsteiner, K, and van der Scheer, J J Exper Med **45** 465, 1927

51 Robinson, E M Eleventh and Twelfth Report, Director, Vet Educ & Res, Dept Agric, Union of South Africa, September 1926, p 9

52 Rosenthal, F Med Klin **26** 205, 1930

mode of action of human serum on trypanosomes is analogous to that of a true chemotherapeutic substance. Since he was unable to demonstrate any trypanocidal action *in vitro* he concluded that human serum was not itself trypanocidal but was rather trypanocidogenic. He thought the trypanocidogenic substance of human serum was transformed by ferments of the blood into the essential trypanocidal material in a manner somewhat analogous to the supposed mode of action of the trypanocidal chemical atoxyl. Atoxyl, in its usual form, is not lethal for trypanosomes *in vitro*, but after injection into the animal it is, in some fashion, transformed into an active product. Ehrlich⁵³ considered that a reduction of the chemical occurred in the animal body, and substances have been demonstrated in the blood⁵⁴ and liver cells⁵⁵ which are capable of effecting such a change of atoxyl in the test tube.

In an effort to demonstrate ferments which might be responsible for the transformation of the trypanocidogenic substances of human serum into the essential trypanocidal material, Rosenthal and Spitzer⁴⁸ blocked the reticulo-endothelial system with india ink and removed the spleen of infected rats prior to injection of human serum. Either procedure decreased the effectiveness of the injected serum. When the reticulo-endothelial system was blocked and splenectomy was done prior to the injection of the serum, very little trypanocidal effect was observed. This appeared to round out well the theory of Rosenthal to explain the mode of action of human serum. Briefly, then, according to Rosenthal, human serum seems to contain trypanocidogenic materials which are transformed into trypanocidal substances by ferments arising from or contained in the reticulo-endothelial system of the treated animal. The final loss of effectivity of the human serum in mice repeatedly treated with this substance Rosenthal and Freund²³ would explain by the exhaustion from the reticulo-endothelial system of the ferments necessary for this conversion. As yet, however, Rosenthal has not been able to demonstrate the existence of substances which can modify the human serum *in vitro* in a manner analogous to that seen with atoxyl.

Recently, however, Handler's³¹ work has thrown some question on the mechanism of the trypanocidal action as advanced by Rosenthal. He has been able to produce "exhaustion" with a human serum which had been heated at 64 C for an hour in order to destroy its efficacy as a trypanocide. Handler explains the loss of effectiveness of human serum in animals which have been repeatedly treated with the substance on the basis of specific antibody production. This he has been able to show rather conclusively by injecting an antihuman serum from the

53 Ehrlich, P. *Verhandl d deutsch dermat Gesellsch* **10** 52, 1908

54 Yamanouchi, T. *Compt rend Soc de biol* **68** 120, 1910

55 Levaditi, C. *Ann Inst Pasteur* **28** 604, 1909

rabbit into trypanosome-infected mice simultaneously with an active trypanocidal human serum. When a considerable amount of a potent antiserum is employed, the trypanocidal activity of the human serum can thus be wholly overcome. Zimmermann⁵⁶ also has opposed the exhaustion theory since by increasing the amount of human serum injected into an "exhausted" animal—thereby perhaps supplying sufficient antigenic material to neutralize the corresponding antibody present in the mouse circulation—trypanocidal effects could be elicited.

One of the important points on which the "exhaustion theory" of Rosenthal was based was his belief that human serum was without activity *in vitro*. Recent work, however, has shown conclusively that no support can be given the exhaustion theory from this quarter. Following the earlier work of Schein,⁵⁷ Terry⁵⁸ and others, Yorke, Adams and Murgatroyd⁴⁶ perfected a method for maintaining pathogenic trypanosomes alive quantitatively *in vitro* at 37 C for twenty-four hours. In contrast to the earlier workers, they found that human serum is highly trypanocidal *in vitro*, dilutions as great as from 1:5,000 to 1:25,000 often being active against a strain of *T. equiperdum* as well as against an old strain of *T. rhodesiense* which they employed. Apparently, there exists no essential difference between the mode of action of the human serum *in vitro* and *in vivo*, and the hypothesis of specific ferments for modifying the human serum after its injection into an animal is groundless. As with the tests in the animal body, no action of human serum on *T. gambiense* is demonstrated *in vitro*, and serums from persons suffering from certain pathologic processes (amebic abscess of the liver and obstructive jaundice) show little or no trypanocidal power by the *in vitro* test. The same workers⁵⁹ have made the interesting observation that the normal serums of certain sheep and goats are naturally antitrypanocidal since often a trypanocidal human serum becomes wholly inactive, as determined by the *in vitro* test, after being mixed with a small amount of the serum of a given sheep or goat.

Prior to this publication by Yorke and his group on *in vitro* action of human serum, the only statement of the successful use of human serum *in vitro* was that by Saito⁶⁰ in 1927. Laveran and Mesnil,¹⁴ Goebel^{8a} and Rosenthal⁶¹ had failed to obtain the trypanocidal effect outside the animal body. Since the publication by Yorke and his group,

56 Zimmermann, G. *Zentralbl. f. Bakt. (Abt. 1)* **120** 422, 1931.

57 Schern. *Arb. a. d. k. Gsndhtsamte* **38** 338, 1911.

58 Terry, B. T. *Proc. Soc. Exper. Biol. & Med.* **9** 41, 1911.

59 Yorke, W., Adams, A. R. D., and Murgatroyd, F. *Ann. Trop. Med.* **24** 115, 1930.

60 Saito, M. *Fukuoka-Ikwadagaku-Zasshi* **20** 52, 1927.

61 Rosenthal^{22b} 30, 52.

numerous workers in different laboratories have reported positive results in in vitro tests and, although the test requires extremely delicate adjustment and tedious attention to details, there remains no question of the success of the method⁶² Adams²⁵ has noted not only with human serum but with the serums of different mammals, birds and reptiles an in vitro trypanocidal effect on the gut and salivary gland forms of *T. gambiense* recovered from laboratory-infected tsetse flies

Serum-Fast Strains of Trypanosomes—As was stated in a foregoing paragraph, when a trypanosome-infected mouse has been treated repeatedly with human serum, the trypanosomes become fast or resistant to the human serum. In mice the parasites finally will resist as much as 2 cc of fresh human serum⁶³. The fastness acquired by the trypanosomes is apparently relatively permanent since the resistant character is retained by the parasites even after passage through the insect vector⁶⁴. In this respect, serum-fastness resembles the resistance which can be developed in trypanosomes against certain drugs⁶⁵. After repeated passage of the trypanosomes through normal animals, the acquired serum-fastness is gradually lost. For example, three races of *T. Brucei* which had become resistant to 2 cc of the serum of either the baboon or man lost their refractory state after from two to fifteen passages through animals⁶⁶. It has been feared by some that serum-fast strains might prove infective for laboratory workers, and one case of what appears to have been a laboratory infection with *T. Evansi* has been recorded⁶⁷. Nevertheless, Collier⁶⁸ inoculated himself, then four other persons, with a serum-fast strain of *T. Brucei* but obtained infection in no instance. Likewise, Mesnil and Leboeuf⁶⁹ found that a baboon remained completely resistant to a strain of trypanosomes which was made fast to baboon serum.

Neither the "exhaustion" hypothesis of Rosenthal and Freund²³ nor the antibody production per se suggested by Handler³¹ explains fully the mechanism of serum-fastness. It appears that the parasites themselves become biologically altered, particularly since the character

62 Fairbairn, H. *Ann Trop Med* **27** 185, 1933. Corson, J. F. *J Trop Med* **36** 365, 1933. Zimmermann⁵⁶. Culbertson and Strong²⁸.

63 Jacoby, M. *Med Klin* **5** 252, 1909. Jacoby²⁴.

64 Lester, H. M. O. *Ann Trop Med* **26** 525, 1932. Corson, J. F. *J Trop Med* **36** 378, 1933, **37** 113, 1934.

65 Duke, H. L. Interim Report, League of Nations International Committee on Human Trypanosomiasis, Boston, World Peace Foundation, 1927, p. 24. Yorke, W., Murgatroyd, F., and Hawking, F. *Brit M J* **1** 176, 1933.

66 Leboeuf, A. *Ann Inst Pasteur* **25** 882, 1911.

67 Mesnil, F., and Blanchard, M. *Bull Soc path exot* **7** 196, 1914. Jacoby⁶³.

68 Collier, W. A. *Arch f Schiffs- u Tropen-Hyg* **28** 484, 1924.

69 Mesnil, F., and Leboeuf, A. *Compt rend Soc de biol* **72** 505, 1912.

of fastness persists during passage through successive vertebrate or invertebrate hosts. Evidence of similar fastness to specific serum antibodies has been presented by Massaglia⁷⁰ in experimental trypanosome infections of guinea-pigs, as well as by Novy and Knapp⁷¹ in experimental infections with the spirochete of relapsing fever in rats.

RELATIONSHIP BETWEEN THE TRYPANOCIDAL ACTION OF HUMAN
SERUM AND MAN'S SUSCEPTIBILITY TO INFECTION
BY TRYPANOSOMES

It was believed by the early workers that the natural immunity of man to the trypanosomes pathogenic for animals was related to the trypanocidal power of human serum⁷². Similarly, the natural immunity of the baboon to infection with all species of trypanosomes, including those infective for man, was explained as being due to the trypanocidal substances of the baboon serum^{3a}. Rosenthal,⁷² on the other hand believing the normal serum itself not to be trypanocidal but rather after injection to give rise by metabolic digestion in the mouse to trypanocidal substances, considered man's immunity due to some other agency. The older point of view has been championed anew by Yorke and his co-workers⁵⁰ largely on the basis of results with their *in vitro* technic.

In support of his teleologic point of view Yorke⁵⁰ has offered an interesting hypothesis concerning the relationship of the human trypanosomes to the trypanosome of nagana, *T. Brucei*. He has suggested, as did others before him,⁷³ that the human forms are modifications of *T. Brucei* and has postulated that if man suffers with some hepatic dysfunction or exists for a time on a diet deficient in essential accessory factors, he may become susceptible to infection with *T. Brucei* because the trypanocidal property disappears from his blood. Once established in such a person, the parasite may be successfully transferred to new human hosts by the natural vector, the tsetse fly. If the fly is of the species *Glossina palpalis*, the trypanosome becomes modified somewhat further than if the vector is of the species *Glossina morsitans*, since the former abounds near the dwellings of man and effects more frequent human passage of the parasite. A trypanosome which represents greater modification from the animal parasite *T. Brucei* is believed by

70 Massaglia, M. A. *Compt rend Acad d sc* **145** 572, 1907

71 Novy, F. G., and Knapp, R. E. *J Infect Dis* **3** 291, 1906

72 Laveran^{3a} Jacoby⁶³

73 Kinghorn, A., and Yorke, W. *Ann Trop Med* **6** 1, 1912. Yorke, W., and Blacklock, B. *Brit M J* **1** 1234, 1914. For a point of view opposite those in the foregoing references see Kleine, F. K., and Eckard. *Ztschr f Hvg u Infektionskr* **75** 118, 1913. Kleine, F. K. *ibid* **77** 184, 1914. Taute. *Arb a d k Gsndhtsamte* **44** 102, 1913. Fischer. *Arch f Schiffs- u Tropen-Hvg* **17** 621, 1913. Corson, J. F. *Ann Trop Med* **26** 109, 1932.

Yorke to be *T. gambiense*, commonly spread by *G. palpalis*, the human pathogen of lesser modification, *T. rhodesiense*, is spread by *G. morsitans*, which is also the vector of *T. Brucei*. Such a hypothesis seems to receive some substantiation from the character of *T. rhodesiense*, already referred to—namely, its greater susceptibility, in comparison with *T. gambiense*, to the action of human serum after repeated passage through laboratory animals.⁷⁴ Yet there are significant experiments which have led to conclusions which oppose the point of view that the trypanocidal activity of serum is responsible for man's immunity against the animal trypanosomes. The serum of patients suffering with trypanosomiasis is as active on the trypanosomes pathogenic for rats as is that of normal persons.⁷⁵ Some strains of guinea-pigs and white rats have been found resistant to *T. Brucei* despite the absence of trypanocidal substances from their blood.⁷⁶ A strain of *T. rhodesiense* which was rendered susceptible to human serum by repeated passage through laboratory animals was shown to retain its infectivity for man^{10d} and, conversely a strain of *T. Brucei* which was rendered serum-fast by repeated exposure to human serum was found unable to infect man.⁶⁸ Finally, because of the widespread distribution of the serum-resistant trypanosome, *T. Lewisii*,¹⁵ among wild rats and its natural spread by means of the rat flea (*Ceratophyllus fasciatus*), which is known to carry certain infectious agents (e. g., *Pasteurella pestis* and the plague bacillus) from the rat to man, the potential incidence of human infection with this rat parasite would seem to be considerable. However, despite its resistance to human serum, *T. Lewisii* is apparently not infective for man, since only a single authentic case of human infection with the parasite had been reported up to 1933.⁷⁶ Because of the reasons given I am inclined to agree with Adams⁷⁷ that the immunity of man to the trypanosomes pathogenic for animals depends on other factors in addition to the trypanocidal activity of the serum.

RELATIONSHIP OF THE AMOUNT OF THE TRYPANOCIDAL SUBSTANCE IN HUMAN SERUM TO HUMAN DISEASE

Although the trypanocidal titer of normal human serum varies considerably, marked reduction or complete loss of trypanocidal activity accompanies certain diseases. In 1907, Ehrlich^{38a} noticed that a disturbance of the liver led to a decrease in the amount of the trypanocidal substance of human serum, and Laveran and Nattan-Larrier,^{9a} in a few trials, reported that variations from the normal strength of

74 Mesnil and Ringenbach^{10c} Corson¹¹ Laveran¹²

75 Corson, J. F. *J. Trop. Med.* **36** 53, 1933

76 Johnson, P. D. *Tr. Roy. Soc. Trop. Med. & Hyg.* **26** 467, 1933

77 Adams, A. R. D. *Ann. Trop. Med.* **27** 309, 1933

the substance occurred in the serums of persons suffering from tuberculosis or syphilis. Beginning with the work of Rosenthal and Kleeman^{40a} in 1915, numerous investigations of the effects of various diseases on the amount of trypanocidal substance in the serum have been carried out. Some workers have reported a decrease in the trypanocidal potency of the serum in infectious processes, particularly when accompanied by fever,⁷⁸ usually with a return to normal on recovery,⁷⁹ but others have failed to note such modifications.⁸⁰ A sharp reduction in serum taken at the time of paroxysm from patients with dementia paralytica who were artificially infected with malaria has been reported,⁸¹ although the serums of similar patients not so treated are said to be particularly active in trypanocidal power.⁸²

A serious difficulty in the interpretation of the results of the various workers is caused by the fact that often sufficient care has not been taken to test all serums under entirely comparable circumstances. It has been shown recently, for example, by Lester⁸³ and by Handler⁸¹ that the potency of serums falls rapidly in the first few days after bleeding. In comparative titrations, therefore, the interval between taking and testing the serum sample must be standardized. It is all too obvious in some cases that this necessary precaution has not been observed.

Liver Disorders—It appears well established that parenchymal injury to the liver tissue causes a decrease in the amount of trypanocidal substance.⁸⁴ Munter^{84c} believed the extent of injury to the liver was quantitatively correlated with the reduction in the amount of trypanocidal substance. According to Platau,^{38b} circumscribed infection of the liver had no effect on the amount of the substance, and hepatic processes going on without icterus affected the amount of the trypanocidal substance only near their terminal stages. There is general agreement that the cure of the disorder leads to the prompt return of the trypanocidal power.⁸⁵ Rosenthal and Nossen³⁹ were able to differentiate, by the trypanocidal serum test, catarrhal icterus, which

78 Neumark, E, and Pogorschelsky, H. *Ztschr f Kinderh* **40** 535, 1926

79 Barlowi, cited by Peutz^{84b}

80 Leichtentritt, B. *Ztschr f d ges exper Med* **29** 658, 1922

81 Jaffe, R. H., and Brown, S. *Proc Soc Exper Biol & Med* **19** 658, 1922

82 Plaut. *Ztschr f d ges Neurol u Psychiat* **101** 512, 1926

83 Lester, H. M. O. *Ann Trop Med* **27** 361, 1933

84 (a) Ehrlich, P. *Beitrage zur Pathologie und Chemotherapie*, Leipzig, A. Pries, 1909. (b) Peutz, L. A. *Nederl tijdschr v geneesk* **66** 1544, 1922. (c) Munter, F. *Klin Wchnschr* **4** 1967, 1925. Rosenthal and Krueger^{8b} Rosenthal and Freund²⁷ Ehrlich^{38a} Platau^{38b} Rosenthal and Kleeman^{40a} Eufinger et al⁴¹ Rosenthal⁵² Yorke et al⁵⁹

85 Platau^{38b} Rosenthal and Nossen³⁹

eliminated the trypanocidal power, from hemolytic icterus, which caused no loss of the substance Yorke and his co-workers⁵⁹ demonstrated in vitro the loss of trypanocidal power in persons with amebic abscess of the liver and obstructive jaundice Ziess⁸⁶ observed that icteric human serum is reactivated by an addition of normal human serum and suggested that the injection of normal human serum might be of help in cases of icterus Support for the suggestion is as yet not available

Tuberculosis—Laveran and Nattan-Larrier^{9a} found that serum from tuberculous patients in amounts of 1 cc inoculated intraperitoneally with the infective dose of trypanosomes delayed the death of mice in two cases till the eleventh and the fourteenth days, respectively, whereas the untreated control animal died on the fifth day after inoculation It seems unlikely from this that the trypanocidal power is significantly reduced in infections with the tubercle bacillus since normal serum inoculated in a similar amount often will inhibit infection for no longer period Yet Laveran's conclusions are in agreement with the later work of Mignoli,⁸⁷ who noted further that the serum of tuberculous persons in progressive stages of recovery manifested increasing trypanocidal activity The serums of two patients with Hodgkin's disease, which by some is believed caused by the avian form of the tubercle bacillus, showed no less activity against *T. Brucei* than did normal serum⁸⁸

Syphilis—The results obtained with the serums of syphilitic patients are especially confusing since there is no consistent evidence of a marked alteration in the trypanocidal power one way or another as the result of syphilitic infection Laveran and Nattan-Larrier,^{9a} using a mixture of trypanosomes and 1 cc of human serum from a syphilitic patient, obtained no evidence of infection in a mouse sixteen days after inoculation, whereas the control mouse not given serum was dead seven days after the inoculation Recently, Adams⁷ was able to effect complete cure in one instance, a result duplicated by him with no other human serum The same author has found that the serum of a syphilitic patient infected also with malaria retarded the appearance of the parasites only four days, i e, the serum apparently had lost practically all its trypanocidal power (cf Jaffe and Brown⁸¹) The trypanocidal substance is said to appear precociously in congenitally syphilitic infants⁸²

Human Trypanosomiasis—The effect of trypanosomiasis on the strength of the trypanocidal substance is of importance with respect

86 Ziess, H Arch f Schiffs- u Tropen-Hyg 25 302, 1921

87 Mignoli, A Riforma med 40 577, 1924

88 Ziess, H Arch f Schiffs- u Tropen-Hyg 25 211, 1921

to the significance of the substance in the protection of man. Some workers have reported that both during and after recovery from trypanosomiasis the serum of patients exhibits no trypanocidal action^{9b}. Recently, however, Corson¹¹ in tests on a strain of *T. rhodesiense* which had been passed repeatedly through mice obtained about as consistent action with the serums of patients infected with trypanosomes as with the serums of normal persons. If Corson's¹¹ observations are confirmed, it follows that the trypanocidal substance of the serum alone is insufficient to account for man's immunity to the animal trypanosomes.

Pernicious Anemia—Frequently in cases of pernicious anemia the trypanocidal substance is reduced in amount⁸⁹. Rosenthal⁸⁹ believed this reduction due to some secondary involvement related perhaps to impairment of normal hepatic function. When the condition of the blood becomes normal, the trypanocidal substance also returns to its normal potency.

Hemophilia—Considerable variation in results occurs in work with the serum of hemophilic persons. Opitz and Zweig⁹⁰ found that the serums of both hemophilic children and their mothers were deficient in the trypanocidal material. Leichtentritt⁹¹ also observed that hemophilic persons showed such a deficiency. Later, Leichtentritt and Opitz,⁹² in a study on the serums of the members of a hemophilic family, found that of eight manifest bleeders the serums of seven showed no trypanocidal activity and that the serums of eighteen of nineteen nonbleeding male and female relatives likewise had no effect on infected mice. In direct contrast to these findings, however, is the work of Weiner and Hartman,⁹³ who found that neither the plasma nor the serum of hemophilic persons or of their mothers and fathers was deficient in trypanocidal activity. Leder,⁹⁴ in a later study, reported that the serums of manifestly hemophilic persons were without trypanocidal power, whereas the serums of carriers were normal. Leder⁹⁴ believed, however, that a means of diagnosing the carrier condition might be worked out through the test for trypanocidal substance. The relation of hemophilia to the presence of trypanocidal substance is worthy of more investigation, since a means of diagnosing the carrier condition would have practical application.

89 Rosenthal, F. *Klin. Wchnschr.* **8** 1436, 1929. Freund and Gassman^{29b} Rosenthal⁵²

90 Opitz, H., and Zweig, H. *Jahrb. f. Kinderh.* **107** 155, 1924.

91 Leichtentritt, B. *Klin. Wchnschr.* **4** 1899, 1925.

92 Leichtentritt, B., and Opitz, H. *Med. Klin.* **23** 59, 1927.

93 Werner, O., and Hartman, E. *Med. Klin.* **22** 1803, 1926.

94 Leder. *Munchen med. Wchnschr.* **75** 562, 1928.

Food Deficiency Diseases—In 1922, Leichtentritt and Zielaskowski⁹⁵ observed that the serums of children suffering from Barlow's disease and from other diseases attributable to deficiencies in accessory food factors exhibited a loss of the trypanocidal substances Gurnmandl and Leichtentritt⁹³ since have found that the serums of children suffering from scurvy, rickets and other avitaminoses were without trypanocidal power. When the cause of the disease was eliminated by supplying the material which the diet lacked, the trypanocidal substance reappeared in the circulation. Young children appear especially suited to the study of the effect of particular diseases, especially the avitaminoses, on the trypanocidal power of the serum since children, in contrast with adults, have but little reserve store of the trypanocidal material.

In an effort to relate the trypanocidal substance with a specific factor of the diet, Jungeblut and I have tested crystalline vitamin C, or cevitamic acid, for trypanocidal activity, this substance having previously been shown by Jungeblut and Zwemer⁹⁶ to have the power to neutralize diphtheria toxin as well as the virus of poliomyelitis. The substance was, however, without trypanocidal activity under the conditions tested (5 mg of cevitamic acid injected intravenously or 10 mg intraperitoneally into mice for three days before infection and on each day during infection until death). The experimental mice and the control mice died after the same average number of days.

Clinical Use of the Test for the Presence of Trypanocidal Substance—Munter^{84c} believed that the degree of reduction of the trypanocidal substance in the serum so closely paralleled the extent of injury to the liver that measurement of this activity carried diagnostic and prognostic significance. Munch,⁹⁷ however, while believing the phenomenon should not be dispensed with, felt the method could hardly be used as a standard for the diagnosis of liver dysfunction. He pointed out that considerable variation occurred both in the action of normal human serums on a given strain of trypanosomes and in the resistance which different strains of trypanosomes manifested to a given human serum. Further Munch⁸⁷ considered that a delay of from eight to fourteen days in performing the test permitted no general adoption of the method. He pointed out that methods already in use were either preferable to the test for trypanocidal substance or were at least as satisfactory for clinical purposes.

95 Leichtentritt, B., and Zielaskowski, M. *Jahrb f Kinderh* **98** 310, 1922

96 Jungeblut, C. W., and Zwemer, R. L. *Proc Soc Exper Biol & Med* **32** 1229, 1935

97 Munch, H. *Munchen med Wchnschr* **70** 945, 1923

Certainly the objections of Munch⁹⁷ were well taken. In view of the apparently diverse diseases which affect the amount of trypanocidal substance, it seems likely the method cannot be generally adopted unless it is much further simplified. Yet work already reported by Rosenthal and his co-workers, Leichtentritt and his group, Mignoli and Yorke suggests that further investigations of possible clinical applications of the test for trypanocidal substance are indicated. Leichtentritt and Zielaskowski⁹⁸ suggested that, by aid of the trypanocidal serum test, a better understanding of the classification of diseases as well as something of the etiology of diseases the causes of which are obscure might be obtained.

RELATIONSHIP OF THE TRYPANOCIDAL SUBSTANCE TO THE BACTERICIDAL AND THE VIRUCIDAL PROPERTIES OF HUMAN SERUM

Normal human serum exhibits a destructive action on many micro-agents of disease. This action was first noted with respect to bacteria and later with respect to trypanosomes. In recent years, the normal human serum has been shown to possess the power to neutralize several of the filtrable viruses which attack man, particularly those of poliomyelitis⁹⁸ and herpes⁹⁹. It seems not impossible that the agent responsible for this property of the serum is a single entity with the ability to manifest its action on a broad diversity of infectious agents. If this is true, exposure to the specific agent, as suggested by Aycock¹⁰⁰ and Aycock and Kramer,¹⁰¹ appears unnecessary for the development of the neutralizing substance in the serum. Aycock has suggested that the immunity of adults and older children to poliomyelitis is built up by the same mechanism as that widely accepted in regard to diphtheria, namely, by exposure to a widespread or even ubiquitous disease agent and by subclinical or aborted attacks of the disease. Aycock's view has been substantiated by numerous workers¹⁰². Similar immunization to trypanosomes by subclinical or unrecognized attacks seems almost impossible, yet the trypanocidal substance is present universally in the blood of healthy persons. Moreover, it increases in frequency with advance of age and is present in various quantities in the serums of different per-

98 Anderson, J. F., and Frost, W. H. *J. A. M. A.* **56** 663, 1911.

99 Flexner, S. *J. A. M. A.* **81** 1785, 1923. Zinsser, H., and Tang, F. F. *J. Immunol.* **17** 343, 1929. Andrewes, C. H., and Carmichael, E. A. *Lancet* **1** 857, 1930.

100 Aycock, W. L. *J. A. M. A.* **87** 75, 1926, *Am. J. Hyg.* **8** 35, 1928.

101 Aycock, W. L., and Kramer, S. D. *J. Prev. Med.* **4** 189, 1930. Kramer, S. D., and Aycock, W. L. *Proc. Soc. Exper. Biol. & Med.* **29** 98, 1931.

102 Fairbrother, R. W., and Brown, W. G. S. *Lancet* **2** 895, 1930. Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B. *J. Prev. Med.* **4** 463, 1930. Brodie, M. *J. Immunol.* **26** 337, 1934, **27** 395, 1934, **28** 1, 1935.

sons If a single substance is responsible for all of these destructive effects, the immunization theory alone is insufficient to explain its presence in the blood of man

Some evidence for the nonspecificity of the "ant substance" in serum has appeared in the literature Gordon and his co-workers,¹⁰³ in an extensive series of papers, which support an earlier point of view of Muir and Browning¹⁰⁴ on the bactericidal activity of blood, have considered that the bactericidal antibody is a nonspecific substance Differences in the effect of serum on various species of bacteria are thought related not to the presence of an antibody for one and the absence of an antibody for another, but to the relative susceptibility of the several species of bacteria to the single nonspecific antibody of the serum The work of Mackie and Finkelstein,¹⁰⁵ on the other hand, indicates that the bactericidal antibody is specific for the particular organism acted on In an effort to explain the results of Gordon, Finkelstein¹⁰⁶ has postulated the existence of a nonspecific factor in suspensions of heated bacterial cultures which inhibits the bactericidal activity of serum and which obscures the specific nature of the absorption of bactericidal substance by bacterial antigens

Weyer¹⁰⁷ has attempted to show that a single "pan-immune body" in normal human serum neutralizes the two viruses poliomyelitis and herpes The work of others, however, chiefly Gay and Holden,¹⁰⁸ indicates no correlation between the neutralizing antibodies against these two disease agents

Strong and I²⁸ have attempted to relate the trypanocidal with the bactericidal substances of human serum We found it possible to remove the trypanocidal property simultaneously with the bactericidal activity by absorption of a serum with a mass of bacteria or, less satisfactorily, with a mass of trypanosomes In a test on a single serum supplied by me, from which both the bactericidal and trypanocidal substances had been absorbed, Jungeblut¹⁰⁹ noted, however, that the poliocidal power was not significantly reduced¹¹⁰ Similarly, according to the results of preliminary tests by Strong,¹¹¹ such absorption fails to remove

103 Gordon, J J Path & Bact **37** 367, 1933 Gordon, J, and Carter, H S *ibid* **35** 549, 1932

104 Muir, R, and Browning, C H J Path & Bact **13** 76, 1909

105 Mackie, T J, and Finkelstein, M H J Hyg **32** 1, 1932

106 Finkelstein, M H J Path & Bact **33** 359, 1933

107 Weyer, E R Proc Soc Exper Biol & Med **30** 309, 1932

108 Gay, F P, and Holden, M J Infect Dis **53** 287, 1933

109 Jungeblut, C W Personal communication

110 It is perhaps significant to note in this connection that Jungeblut has been able to absorb the poliocidal substance from human serum by contact *in vitro* with a suspension of red cells (J Immunol **27** 17, 1934)

111 Strong, P S Personal communication

the activity of the serum against the virus of herpes. This seems to indicate that the trypanocidal substance is more closely related to the bactericidal than to the virucidal substances.

A different relationship, quite as deserving of consideration, but especially difficult to explain on the basis of specific immunization, can also be pointed out. The trypanocidal and virucidal activities are exhibited almost exclusively by the serum of man, whereas the bactericidal property is manifested by the blood of many species of animals. It is generally stated that the serums of many normal adults fail to show activity against the viruses of herpes or poliomyelitis, while the trypanocidal substance is always present in healthy persons after the first few months of life. However, these differences are probably only quantitative, the failure to find some trace of virucidal activity in so-called negative serums being due to insufficient delicacy of the means of testing. From this point of view, the trypanocidal activity seems more closely related with the virucidal than with the bactericidal activity of the serum.

Because of the confusion in which these parallelisms and contrasts remain, it is impossible as yet to draw conclusions on the identity or difference between that substance responsible for the trypanocidal activity of human serum and those responsible for the bactericidal and virucidal actions. The question is certainly one of fundamental significance in immunology, but its solution must await the fruits of the extended investigation which surely the future will bring.

SUMMARY

The normal serum of man will destroy the trypanosomes pathogenic for animals (e g, *T. Brucei*, *T. equiperdum*, *T. equinum*, etc.) either in the test tube or in the body of rodents infected with these parasites. Human serum does not affect the trypanosomes pathogenic for man (*T. gambiense*, *T. rhodesiense* or *T. Cruzi*), the common trypanosome of rats (*T. Lewisii*) or a trypanosome of newts (*T. diemyctyli*). One of the human trypanosomes, *T. rhodesiense*, is distinctive in that it becomes susceptible to the action of human serum after it has been passed successively through mice. The serum of no other animals, excepting certain monkeys, manifests trypanocidal activity. The serum of some monkeys, however, e g, that of the baboon, destroys not only the trypanosomes pathogenic for the lower animals but, as well, those infective for man.

The trypanocidal substance of human serum is found in the globulin fraction of the serum. It is thermolabile, being destroyed wholly when the serum is heated at 64 C. for an hour, and being reduced rapidly when the serum is let stand at room temperature. The trypanocidal

substance passes readily through Berkefeld filters and with diminished intensity through collodion ultrafilters. It is removed from a serum by absorption with trypanosomes or bacteria (the typhoid bacillus, *Proteus*). The substance exhibits its activity independently of all the known components of alexin. The essential substance in the human serum which brings about the trypanocidal effect is antigenic, and a specific anti-trypanocidal antibody develops in rabbits repeatedly treated with an active serum.

The trypanocidal substance probably originates in the normally functioning healthy liver. It is found in the blood serum and in serous exudates. The cerebrospinal fluid and the urine are without trypanocidal power. The substance appears in infants at a very early age and may be present at birth. It is probably elaborated within the body of the young child, since it is found neither to pass the placenta nor to occur in human milk. The trypanocidal power is enhanced in women late in the period of gestation and is maintained at a high level for some time after delivery.

It seems unlikely that the action of the trypanocidal substance is that of an opsonin or an agglutinin, and the property is manifested wholly without the intervention of alexin. Some investigators have felt it acts essentially as a chemotherapeutic substance. Susceptible strains of trypanosomes become resistant or fast to human serum after repeated exposure to the serum in a manner comparable to that in which they become resistant or fast to drugs.

Since human serum affects only those species of trypanosomes which are pathogenic for animals and which are noninfective for man, and is without effect on the trypanosomes which are infective for man, it is by some believed that the trypanocidal action of the serum is responsible for man's immunity to the animal pathogens. It is known, however, that strains of animal trypanosomes which have been rendered serum-fast still are noninfective for man, and that strains of human trypanosomes (*T. rhodesiense*) which become susceptible to human serum after repeated passage through animals retain their infectivity for man. Furthermore, the serum-resistant parasite *T. Lewisi*, which is widespread among rats, is apparently unable to infect man. The serum of patients with trypanosomiasis is as active in trypanocidal power as that of normal persons. It appears, therefore, that the immunity of man to the animal trypanosomes depends on factors other than the trypanocidal activity of the serum.

The trypanocidal activity of human serum is sharply reduced in diseases which cause extensive destruction of the parenchyma of the liver. Less conclusive evidence of alteration in the potency of the substance has been offered in other infectious diseases (e.g., tuberculosis and syphilis) and in those attributable to deficiencies in accessory food fac-

tors No significant reduction is observed in human trypanosomiasis. It seems possible, from the observations of some workers, that the carrier condition in hemophilia can be identified by the absence of the trypanocidal substance from the serum.

It appears not unlikely that trypanosomes are but one form of infectious agent on which a single destructive entity in human serum acts. The fact that both the trypanocidal and bactericidal substances are removed from the serum by absorption with either trypanosomes or bacteria points toward a close similarity between the trypanocidal and bactericidal powers. The trypanocidal activity, however, differs from the bactericidal property and resembles the virus-neutralizing function of human serum in being limited to the serum of man and a few closely related primates and in occurring without the presence of alexin. If further study indicates that these effects are all manifestations of a single entity of the serum or, what seems more likely, that they arise in analogous manners, support for the assumption that these "antistances" arise by immunization through contact with the specific antigen is difficult to maintain, since at no time in life does the average person suffer subclinical or abortive infection with trypanosomes.

Notes and News

University News, Promotions, Resignations, Appointments Deaths, etc

—George B McGrath, medical examiner of Suffolk County (Boston) since 1907 and professor of legal medicine in the Harvard University Medical School since 1931, has resigned because of ill health. He will be succeeded as examiner by William J Brickley, associate examiner.

Edwin Raymond LeCount, professor of pathology and chairman of the department of pathology at Rush Medical College, died on Aug 23, 1935, at the age of 67.

Max Pinner, Desert Sanatorium, Tucson, Ariz., has been appointed principal diagnostic pathologist to the hospitals for tuberculosis of the state department of health, New York.

Gustav Hauser, professor emeritus of general pathology and morbid anatomy in the University of Erlangen, has died at the age of 79.

Bela Halpert, assistant professor of pathology and surgery in Yale University, has been appointed head of the division of pathology and associate director of laboratories in the Jewish Hospital of Brooklyn.

John I Fanz, professor of pathology in Temple University, has died at the age of 44.

Neil McLeod, instructor of pathology in the University of Pennsylvania, has died of injuries received in an automobile accident.

Lydia Rabinovitch Kemper, who was the only woman co-worker of Koch and for many years director of the bacteriologic institute of the Moabite (now Koch) Hospital, Berlin, died on August 5 at the age of 64. She was instructor in bacteriology in the Woman's Medical College in Philadelphia in 1896-1899.

Alexander C Abbott, professor emeritus of hygiene and bacteriology in the University of Pennsylvania, has died at the age of 75.

Charles Norris, chief medical examiner of New York City since 1918, died on Sept 11, 1935, at the age of 67.

Awards—The Sedgwick Memorial Medal of the American Public Health Association has been awarded to Haven Emerson, professor of public health administration in Columbia University.

The medical faculty of the University of Bern, Switzerland, has awarded a prize of 1,000 Swiss francs to Leslie T Webster, of the Rockefeller Institute for Medical Research, for his investigations in the field of encephalitis.

Fund for the Study of Dementia Praecox—According to *Science* a fund of \$40,000 has been donated by the Supreme Council, Scottish Rite Masons, for the study of dementia praecox. The research program will be under the direction of the National Committee for Mental Hygiene, New York City. The field representative is Nolan D C Lewis, formerly director of laboratories of St Elizabeth's Hospital, Washington, D C, now director of clinical pathologic research of the Neurological Institute and professor of neuropathology in Columbia University.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

EXPERIMENTAL PRODUCTION OF ANKYLOPOIETIC ARTHRITIS K SONNENBERG,
Virchows Arch f path Anat **293** 724, 1934

By repeatedly injecting horse serum into knee joints of rabbits sensitized to the serum Klinge had been able to produce arthritis deformans. The object of Sonnenberg's work was to determine whether a similar procedure would lead to ankylosing arthritis if the joint was completely immobilized. In each of a series of rabbits a knee joint was immobilized by a surgical procedure. After recovery each animal was sensitized by a single injection of 4 cc of horse serum. Six weeks later a series of from four to six injections of from 1 to 2 cc of the sensitizing serum directly into the immobilized joint was begun. The injections were spaced at intervals of from eight to fourteen days. The animals were killed at varying intervals and the joints examined histologically. The local allergic reaction led to inflammatory union of the synovia with the joint cartilages. Ultimately there resulted fibrous and cartilaginous ankylosis of the joint. From his own and Klinge's work Sonnenberg concludes that allergic inflammation of a mobile hyperergic joint leads to arthritis deformans, that similar inflammation of a completely immobilized joint leads to ankylopoietic arthritis, that allergic inflammation of an incompletely immobilized joint leads to a combination of arthritis deformans and ankylopoietic arthritis, and that immobilization without inflammation never leads to ankylosis.

O T SCHULTZ

PHYSIOLOGY AND PATHOLOGY OF THE CIRCULATION A BIER, Virchows Arch f path Anat **293** 738, 1934

This is a fifty-six page continuation of an article the first part of which appeared in volume 291 of *Virchows Archiv*. By numerous clinical observations and by discussion of them Bier seeks to establish the correctness of his attraction theory of the circulation. He considers the hemodynamics of the circulatory system inadequate to explain the observations cited. An important factor in the maintenance or the reestablishment of local circulation is the need or "hunger" of tissues and organs for fresh blood, required by nutrition and function and for the removal of waste products. By participation of small vessels and capillaries blood is drawn into the tissue by suction in a quantity greater than can be explained by the circulatory mechanics of the heart and large vessels.

O T SCHULTZ

ERYTHROCONTES AND ERYTHROPOIESIS IN PERNICIOUS ANEMIA AND IN THE EMBRYO F ZANATY, Virchows Arch f path Anat **293** 794, 1934

In 1928 Schilling applied the term "erythrocontes" to minute rodlike bodies seen in the polychromatic megal-erythrocytes of pernicious anemia. He at first believed these bodies to be parasites belonging to the bartonella group. More recently he has held that they are probably derived from the constituents of polychromatic megalocytes. In an attempt to determine the nature of erythrocontes and their possible specific relationship to pernicious anemia, Zanaty examined the bone marrow and spleen of persons dying of diseases other than blood dyscrasias and the blood of human embryos of the third to the ninth month, the latter because a macrocytic state of the blood is physiologic in the embryo. Erythrocontes were not seen in any of the material studied, and Zanaty therefore concludes that they have no relationship to the polychromatophilia of either normocytic or macrocytic

blood The failure to discover basophilic stippling of fetal erythrocytes is proof of a fundamental difference between the polychromatophilia of fetal and that of postfetal blood He does not accept the view that pernicious anemia is characterized by a return to a fetal type of erythropoiesis The megaloblasts of pernicious anemia are morphologically different from those of fetal blood Zanaty does not accept Naegeli's view that megaloblasts and macroblasts are distinct types of cells, but holds that they are a single type of cell

O T SCHULTZ

Pathologic Anatomy

TUBEROUS SCLEROSIS WITH UNUSUAL LESIONS OF THE BONES JACQUES S GOTTlieb and GEORGE R LAVINE, Arch Neurol & Psychiat **33** 379, 1935

Clinically tuberous sclerosis is characterized by adenoma sebaceum, convulsive seizures and mental deficiency Any member of the triad may be absent, and there are always neoplasms in some viscera (kidneys, spleen, retina, lungs) In the present case, that of a woman aged 23, mental symptoms were present—the patient was noisy and destructive There was a previous history of epilepsy and idiocy At birth, a reddish-brown patch was present on the right side of the neck This gradually grew larger, and in addition raised white patches and "reddened nodules" appeared on the face (adenoma sebaceum) Tumors in the retina (phakomas) and spina bifida of the sacrum were also found One of the retinal tumors exhibited at its periphery numerous fine capillaries dipping into its substance, and periosteal thickening with general osteoporosis of both metatarsal and metacarpal bones and their phalanges was in evidence Several of the bones of the hands showed areas of marked rarefaction, suggesting cysts There was osteoporosis of the skull

G B HASSIN

FAT TISSUE IN THYROID ADENOMA F BRENNER, Centralbl f allg Path u path Anat **62** 113, 1935

In a portion of thyroid gland, 10 by 8 by 3 cm, five adenomas ranging in size to that of a walnut were encountered Microscopically these were of the micro-follicular type with a partially hyalinized stroma Fat tissue in masses up to 15 mm in diameter occurred near the periphery of the adenomas and were separated from the connective tissue capsule by a band of parenchyma 0.5 mm wide In the larger fat masses the cells lay next to one another and resembled typical fat tissue At the borders of such fields single fat cells occurred between the small alveoli of the adenoma The alveoli exhibited no retrogression changes Replacement of gland tissue by fat is well known in such places as the salivary glands and pancreas, where the fat cells originate in the connective tissue cells of the organ By analogy Brenner believes that in this instance metaplastic changes had occurred in the adenomas, the parenchyma of which was retrogressing

GEORGE RUKSTINAT

AN UNUSUAL ANOMALOUS BAND OF THE LEFT AURICLE K SCHWEIKART, Centralbl f allg Path u path Anat **62** 114, 1935

Anomalous tendinous bands of the left auricle occur seldom as compared with such bands of the right auricle but have a certain uniformity This consists in the attachment of one end to the valvula foraminis ovalis and of the other to a mitral leaflet or to the ventricular endocardium In the present case the attachment to the mitral leaflet only was demonstrable The free end was, however, bifid and matched depressions in the valvula foraminis ovalis Presumably detachment was effected during marked cardiac dilatation in a woman who suffered from nephritis and mitral stenosis with insufficiency Death was due to uremia

GEORGE RUKSTINAT

PATHOLOGIC AND ANATOMIC CHANGES IN SO-CALLED LEPROSY OF RATS R JAFFE
and G KAHLAU, Frankfurt Ztschr f Path **46** 218, 1933

Stefansky in 1903 described a disease in rats which closely resembled human leprosy Jaffe and Kahlau examined the organs of sixty rats in which this disease had been produced experimentally As controls, organs of guinea-pigs which had received injections of leprosy bacilli isolated from human lesions were examined In the rat the disease was characterized by nodules in the skin and lymph nodes Microscopically they consisted of granulation tissue with large cells which contained much cytoplasm and also a number of acid-fast bacilli These cells were referred to as leprosy cells Bacilli were also found in the Kupffer cells of the liver, in the spleen and in the cells of the reticulo-endothelial system in other organs These cells were thought to be the origin of the leprosy cells Later, round cells and giant cells of the Langerhans type were found in addition to the leprosy cells Whereas in the rat the testes were never involved, the testes in man very often showed the characteristic granulation tissue In man the leprosy cells showed many vacuoles while these were not present in those of the rat The characteristic arrangement of the bacilli within the leprosy cell so often seen in man was not found in the rat The authors conclude that the changes in the organs in man closely resemble but are not identical with those in the rat

OTTO SAPHIR

MALFORMATION OF THE LOWER EXTREMITIES (SIRENOMELUS) E HEITS, Frankfurt Ztschr f Path **46** 241, 1933

Heits examined two cases of this anomaly In the first the sacrum and coccyx were missing and only four lumbar vertebrae were recognizable Both iliac bones were horizontally placed and were adherent to each other The ischiatic and iliac bones were also firmly adherent to each other The pubic bones showed no changes There was a rudimentary tibial bone and two patellae In the second instance the changes in the lower extremities were similar to those in the first instance In addition there were revealed thin soft parietal bones and hydrocephalus Also the right thumb was absent Heits concludes that the sirenoformation results from a primary arrest of the development of the caudal segment of the body occurring probably in the first half of the third week of the embryonal development The cause of this malformation is apparently exogenous, for various malformations of other organs may be present

OTTO SAPHIR

MARKED DEFORMITY OF THE BRAIN FROM MENINGEAL CYST D SCHRANZ, Frankfurt Ztschr f Path **46** 252, 1933

In a 50 year old man who committed suicide a cyst was found in the left sylvian fissure The cyst measured 8.5 by 9 cm in diameter and extended from the orbital portion of the inferior frontal gyrus to the lower portion of the central gyrus and reached the temporal gyrus The pia mater over the cyst was markedly thickened and milky white The brain was greatly compressed but did not reveal any other gross or histologic change The history showed absence of trauma but brought out that he always had been left-handed Schranz does not believe that the cyst was congenital, but thinks it developed later in life, possibly as a result of an old healed circumscribed meningitis

OTTO SAPHIR

BRAIN CHANGES IN LEUKEMIA W HAMBURGER, Frankfurt Ztschr f Path **46** 257, 1933

Hamburger examined four cases of myeloid leukemia and one of lymphatic leukemia The hemorrhages in the brain seen in leukemia are grossly different from those due to other causes The former are firmer and are distinctly yellowish brown They are often diffusely present throughout the white substance, are often small and resemble those seen in hemorrhagic encephalitis The capillaries are dilated and often filled to capacity with white blood corpuscles This condition is

spoken of as white stasis. The hemorrhages are the result of diapedesis of the blood corpuscles. Since often most of the cells in the vessels are white blood cells, the hemorrhages may give the erroneous impression of leukemic infiltration.

OTTO SAPHIR

MUCUS-CONTAINING STONES IN THE APPENDIX J VON SOOS, Frankfurt Ztschr f Path 46 286, 1933

An appendix containing 134 round stones covered with and also containing mucus was removed at autopsy from a 69 year old man. The appendix was 8 cm long and spindle-shaped, and its orifice was markedly narrowed. The stones measured from 1 to 3 mm in diameter. In the center of each was a yellow opaque granule. On chemical examination it was shown that the calculi consisted of an albuminous material, phosphates and calcium carbonates. Von Soos believes that the occurrence of mucus within the stone is a secondary process. The patient had been a stone cutter and apparently had swallowed the particles of stone which formed the nuclei of the calculi.

OTTO SAPHIR

NECROSIS OF THE PANCREAS W MANZ, Frankfurt Ztschr f Path 46 295, 1933

Twenty-five instances of acute and subacute necrosis of the pancreas are reported. In the majority of these cases gallstones were found. In some instances fat necrosis and the necrosis of the pancreas were the result of carcinoma which caused either primary pressure necrosis of the pancreatic parenchyma or narrowing of the ducts. Occasionally the disease was brought about by the presence of ascarides within the ducts with resulting severe inflammation. Rarely a primary chronic inflammation of the duct was thought to have caused the necrosis of the pancreas. Very occasionally histologically demonstrable disease of the pancreatic arteries caused the necrosis of the pancreas. Manz does not believe that primary necrosis of the pancreas may occur in the absence of inflammatory changes as was thought by Balo (*Virchows Arch f path Anat* 259 733, 1926).

AUTHOR'S SUMMARY

AMYLOID CONTRACTED KIDNEYS H WILLER, Frankfurt Ztschr f Path 46 306, 1933

Willer reports three cases of uncomplicated amyloid infiltration of both kidneys which had caused contraction of these organs. The ages of the patients were 31, 39 and 42, respectively. They all had general edema and much urinary albumin. The nonprotein nitrogen of the blood was determined for two patients and amounted to 32 mg and 117 mg per hundred cubic centimeters, respectively. The arterial blood pressure was 125 systolic and 75 diastolic in the first, 120 systolic and 75 diastolic in the second and 120 systolic and 80 diastolic in the third patient. The eyegrounds were normal. The autopsies revealed neither arteriosclerosis nor cardiac hypertrophy. The kidneys grossly were smaller than normal. Their surfaces were grayish white to yellow with irregularly outlined retracted areas. The cut surfaces were glossy brownish, and the cortex was markedly reduced in size. Histologically almost every glomerulus showed changes. Most of the glomeruli were enlarged, occasionally one was contracted. They were poor in nuclei and almost completely replaced by a homogeneous substance which gave the characteristic amyloid staining reactions. Bowman's capsules were thickened and contained much newly formed connective tissue, which occasionally extended into the loops of the glomeruli. The tubuli revealed changes which were characteristic of lipoid nephrosis. The interstitial tissue showed many scars and masses of amyloid. The lumens of the smaller arteries and particularly of those of the vasa afferentia were narrowed by rings of amyloid. Also a fourth case is reported. The patient was 77 years old. The arterial blood pressure was 140 systolic and 90 diastolic, and there also was marked albuminuria. The heart was not enlarged. The kidneys were similar

to those seen in the first three instances. Rats in which amyloid contracted kidneys were produced experimentally did not reveal hypertrophy of the heart.

Willer stresses the point that even though in these four instances both kidneys were severely diseased the patients did not show hypertension and the hearts were not hypertrophic at autopsy. This, he believes, speaks against the assumption that renal lesions and arterial changes are the primary factors in hypertension.

OTTO SAPHIR

CONTRACTED SOLITARY KIDNEY. H. WILLER, Frankfurt Ztschr f Path **46** 321, 1933

Willer reports two cases. In one there was agenesis of the left kidney and left ureter and chronic glomerulonephritis in the right kidney. The patient died of uremia. The arterial blood pressure was 190 systolic and 110 diastolic, declining to 155 systolic and 95 diastolic. The nonprotein nitrogen content of the blood was 79 mg per hundred cubic centimeters and increased to 112 mg. There was albumin in the urine. The second patient had an arterial blood pressure of 185 systolic and 100 diastolic. The urine contained much albumin. At autopsy only a minute portion of renal tissue was found in place of the left kidney. The right was an arteriolosclerotic contracted kidney. The minute portion of the left kidney revealed the presence of only a few collecting tubules, neither convoluted tubules nor glomeruli were found. Willer stresses the fact that in spite of the marked decrease of the parenchyma of the kidney in each instance the hypertrophy of the left cardiac ventricle in each instance was relatively slight. He also maintains that the increase in systolic pressure is less than one would expect considering the severe loss of renal tissue. This indicates to him that there is no ratio between parenchymal changes in the kidney, on one hand, and hypertension and hypertrophy of the left cardiac ventricle, on the other.

OTTO SAPHIR

HISTOLOGY OF THE EXTRAMURAL CARDIAC NERVOUS SYSTEM IN CASES OF SUDDEN DEATH. M. I. AWDEJEW ET AL., Virchows Arch f path Anat **293** 351, 1934

The anatomic substrate of suddenly fatal cardiac failure was sought first in the myocardium. Later the intrinsic conduction system of the heart became the subject of investigation. In the study here reported attention was directed to the extramural cardiac nervous system: the vagus nerve and its ganglion and the cervical sympathetic nerve and the superior cervical and stellate ganglions. The material, which was investigated chiefly by means of a modified Bielschowsky method, was derived from thirty-one cases of sudden death which had been subjected to medico-legal necropsy. Twenty-four were cases of coronary arteriosclerosis with the usual concomitants of this condition, namely, thrombosis, myomalacia, myofibrosis and cardiac aneurysm with rupture. Four were cases of syphilitic mesaortitis with aneurysm but without rupture, two were cases of marked cardiosclerosis without arteriosclerosis, and one was a case of fatal epilepsy in which the heart and vessels were normal. The ages of the subjects varied from 27 to 85 years. Material from thirteen controls varying in age from 5 to 73 years was also studied. In coronary arteriosclerosis the vagus and sympathetic ganglions revealed a condition of fibrotic shrinkage. This is held to be, not the cause of the arteriosclerosis, but the result of the latter. The alteration to which great importance is attached is globular swelling of the processes of the nerve cells and proliferation of axis-cylinders with the formation of networks of fibrils about and between the groups of ganglion cells. With these changes in the ganglion were associated enlargement, nodular swelling and irregularity in the course of nerve fibrils in the trunk of the vagus. Similar alterations were not observed in the sympathetic ganglions or the sympathetic trunk. The histologic changes observed are held to be the result of a state of hyperirritability of the vagus and its ganglion. To this state are ascribed the attacks of angina pectoris which subjects with coronary sclerosis were known to have had, as well as the sudden stoppage of the heart.

O. T. SCHULTZ

CHANGES IN THE TEETH IN TUMORS OF THE JAW M N ZAJEWLOSCHIN and S I LIBIN, *Virehows Arch f path Anat* **293** 364, 1934

A microseopic study of the teeth was made in fifty-six cases of neoplasm of the jaw. In most of the cases the growth was a primary tumor of the jaw, in some it was a tumor of a contiguous structure that involved the jaw by continuity, in two it was metastatic. There were observed degenerative, inflammatory and neerobiotic changes in the pulp and pericementum and resorptive and appositional alterations in the hard structures of the teeth. Since such changes were noted in teeth at some distance from the tumor they are ascribed, not to direct action of the tumor, but to chemical changes brought about by the tumor. In a rather surprising percentage of the cases of malignant tumor tumor cells were seen in the pulp.

O T SCHULTZ

CHANGES IN THE STERNOCLAVICULAR JOINT IN RELATION TO AGE P LANGEN, *Virehows Arch f path Anat* **293** 381, 1935

Both sternoclavicular joints were subjected to microscopic study in 200 cases. The subjects ranged in age from prematurity to 85 years. The histologic character of the joint at various periods of life is described. At 25 to 30 years of age degenerative and regressive changes in the cartilage make their appearance and are followed by increased vascularity and reorganization of the underlying bone. By the age of 40 years these changes are evident in every person and have resulted in a state which Langen terms arthritis deformans. The changes described, and by inference those of arthritis deformans, lie at the boundary between the physiologic and the pathologic.

O T SCHULTZ

RESISTANCE OF THE GANGLION CELLS IN CERTAIN DISEASES OF THE NERVOUS SYSTEM HANS-JOACHIM SCHERER, *Virehows Arch f path Anat* **293** 429, 1934

Parenchymatous cells in general are more susceptible to harmful agents than supporting cells. The same doctrine is accepted for the nervous system, the ganglion cells of which are most vulnerable. These cells, however, were found to be highly resistant to infiltrating glioblastoma of the brain, to multiple sclerosis and to Wilson's disease in the involved areas of the brain. The vulnerability of the ganglion cells is most evident in vascular disturbances of the brain.

O T SCHULTZ

FATAL PERICARDIAL HEMORRHAGE DUE TO RUPTURE OF A CORONARY ARTERY JANINA KOWALCZYKOVA, *Virehows Arch f path Anat* **293** 464, 1934

Necropsy of an 80 year old man with senile arteriosclerotic gangrene of the foot who died suddenly revealed a massive hemorrhage into the pericardial cavity. The hemorrhage had come from a ruptured sclerotic and atheromatous subepicardial branch of the left coronary artery and was not the result of myocardial necrosis. The author could find no record of a similar spontaneous nontraumatic rupture of a diseased coronary artery.

O T SCHULTZ

Immunology

ANAPHYLACTOGENIC PROPERTIES OF MILK B RATNER and H L GRUEHL, *Am J Dis Child* **49** 287, 1935

The proteins isolated from raw milk are chemically and biologically distinct. There are no antigenic changes as a result of drying, acidification or pasteurization. Dried, acidified, superheated or evaporated milk shows no loss of the antigenic properties of the casein fraction. As a sensitizing agent given by injection

milk that has been evaporated, freshly boiled for several hours or superheated shows practically no loss of the antigenic character of the lactalbumin. As a shock agent by injection evaporated or superheated milk shows an unmistakable loss of the antigenic properties of the whey fraction. This is more marked with evaporated than with superheated milk (smaco 303). Evaporated, freshly boiled and acidified evaporated milks, when fed by mouth, show a marked reduction in sensitizing ability. The loss of the antigenic properties of heated milks is presumably due to coagulation of the whey proteins, for which there is some evidence of reversibility. The further reduction in the antigenic properties of heated milks when fed by mouth is due to the fact that coagulation delays the passage of proteins through the gastro-intestinal tract, thus making for more complete digestion and diminishing the probability of the absorption of native antigens through the intestinal wall. In view of the experimental and clinical observations recorded here it appears that evaporated milk is the modification of greatest value for the person who is sensitive to milk.

FROM THE AUTHORS' SUMMARY

ANAPHYLACTOGENIC PROPERTIES OF MALTED SUGARS AND CORN SYRUP B. RATNER and H. L. GRUEHL, *Am J Dis Child* **49** 307, 1935

Malt extracts and the barley malt from which they are derived are highly anaphylactogenic and animals sensitized to them react specifically to the original protein constituent, hordein, present in barley. Persons who are sensitive to barley may show symptoms after the ingestion of malt extracts and malt brews. Corn syrups and pure dextrimaltose sugars are nonanaphylactogenic. It is improbable that the highly purified and refined sugars have anaphylactogenic properties. The addition of wheat germ or dried milk to nonanaphylactogenic preparations of dextrimaltose converts them into substances which are definitely anaphylactogenic. Persons sensitive to wheat, yeast or milk may have allergic exacerbations from the use of such compound preparations of dextrimaltose. Those who cannot tolerate honey may be sensitive to the specific protein elements of the nectar from which it is derived, for example, buckwheat. Allergy to carbohydrate foods cannot therefore be attributed to the carbohydrates per se but must be ascribed to the protein constituents which are added to certain compound carbohydrate food preparations. The experiments reported make it apparent that pure dextrimaltose, corn syrup and crystalline sugars play no rôle in allergy.

FROM THE AUTHORS' CONCLUSIONS

EFFECT OF ACUTE DISEASES ON THE REACTION OF THE SKIN TO TUBERCULIN A. G. MITCHELL et al, *Am J Dis Child* **49** 695, 1935

A depression of allergy to tuberculin exists during the acute stages of scarlet fever, and probably of measles, which disappears during the convalescent stage. This is shown (1) by significant differences in the occurrence of positive reactions during the acute and convalescent stages, (2) by the depression of allergy in the acute stage in persons known to give positive reactions, (3) by a delay in the time of the appearance of the reaction during the acute stage, and (4) by the smaller size of the reaction in the acute stage as compared with that in the convalescent stage. From a practical clinical standpoint it follows that failure to elicit a skin response to tuberculin during the acute stage of scarlet fever, and probably of measles, cannot be accepted as evidence that the person does not normally give a positive reaction. No statistically significant difference can be demonstrated between the rates of positive reaction to tuberculin during the acute and convalescent stages of diphtheria. This is the more conclusive since stable rates of reaction were obtained for the acute and the convalescent stage of the disease.

FROM THE AUTHORS' SUMMARY

IMMUNITY IN EPIZOOTIC FOX ENCEPHALITIS R G GREEN et al, *Am J Hyg*
21 366, 1935

Recovery from fox encephalitis is accompanied by an acquired permanent immunity, which appears to depend on the presence of an antiviral in the blood stream. The development of the acquired immunity evidently requires several weeks in the most susceptible animals. As fatalities are confined almost entirely to the first week of the disease, acquired immunity probably plays little role in individual recovery. It seems that the degree of natural immunity at the onset of the disease determines recovery from this infection. An active antiviral can be developed in serum by hyperimmunization. A maximum antiviral content is obtained in serum only after more than a year of weekly injections. Such a serum shows its activity under experimental conditions only when mixed with the virus before injection. Delayed infection occurs approximately thirty days after the injection of the serum-virus mixture. The delayed infection is marked by the acute symptoms and the presence of the specific inclusions typical of the natural disease. Delayed infection may be prevented by a second injection of serum three weeks after the injection of the serum-virus mixture. Foxes surviving the injection of the serum-virus mixture and aided by a second injection of serum are generally immune to the disease, but about 8 per cent are still susceptible to experimental infection six months later.

FROM THE AUTHORS' SUMMARY

ALLERGIC REACTIONS OF ACTINOMYCETES D R MATHIESON et al, *Am J Hyg*
21 405, 1935

Infection and immunization with acid-fast actinomycetes tend to produce an allergic sensitization in experimental animals. No cross-sensitization to tuberculin could be demonstrated. Continued immunization leads to desensitization. Normal persons give more frequent and more marked skin reactions to *Actinomyces bovis* than do actinomycotic patients. Whereas single injections of *A. bovis* rarely produce infection, repeated inoculations usually do. This is in agreement with the findings of Nakayama, who first suggested that allergic sensitization is a factor in the etiology of actinomycosis. No sensitization demonstrable by skin tests could be induced in rabbits inoculated with saprophytic aerobic actinomycetes. Representative actinomycetes do not elicit reactions similar to those described by Schwartzman for meningococci and other bacteria.

FROM THE AUTHORS' SUMMARY

IMMUNIZATION WITH FORMOLIZED TISSUE CULTURES OF TYPHUS RICKETTSIA
 I J KILGIER and M ASCHNER, *Brit J Exper Path* **15** 337, 1934

Data are presented showing that it is possible to immunize animals with emulsified tissue cultures of *Rickettsia* sterilized with dilute solution of formaldehyde. Cultures of the European and Mediterranean rat virus were used, and in each instance three injections, equivalent to about one sixth of a guinea-pig's tunica albuginea testis, proved sufficient to induce an effective immunity. Old as well as fresh cultures and freshly formaldehydized suspensions as well as old ones are equally effective for immunization. The failure to induce immunity with dead *Rickettsia* in infected tissues appears to be due to the insufficient amount of antigen present in the infected tissues. It is suggested that this is the reason for a similar failure with viruses, and that vaccines made from virus cultures may offer a solution to the problem of active immunization with dead virus.

FROM THE AUTHORS' CONCLUSIONS

THE PROPERTIES OF THE V₁ ANTIGEN OF THE TYPHOID BACILLUS A FELIX,
 S S BHATNAGAR and R M PITTS, *Brit J Exper Path* **15** 346, 1934

The V₁ antigen of the typhoid bacillus can be demonstrated by the inagglutinability of the living organisms by pure O serum or by their agglutinability with

pure V₁ serum The two methods give equally reliable results The development of V₁ antigen is suppressed when virulent strains of the typhoid bacillus are grown at temperatures between 20 and 25 C and between 40 and 44.5 C The application of this technic to similar studies of other bacterial species is suggested The resistance of the V₁ antigen to heat is described as it is reflected by agglutination and absorption tests and by the formation of antibodies in the rabbit Saline extracts of cultures of virulent strains of the typhoid bacillus contain V₁ antigen, precipitable by pure V₁ antiserum The use of formaldehydized extracts is suggested for the preparation of relatively potent V₁ antiserum

FROM THE AUTHORS' SUMMARY

ANTIGENIC DIFFERENCES BETWEEN RELATED BACTERIAL STRAINS A CRITICISM OF THE MOSAIC HYPOTHESIS F M BURNET, Brit J Exper Path **15** 354, 1934

An extract of the Flexner dysentery bacillus is composed of immunologically similar molecules of antigen and not of the mixture of antigens postulated in the mosaic theory of bacterial antigenic structure

FROM THE AUTHOR'S CONCLUSIONS

THE IMMUNOLOGICAL RELATIONSHIP OF PSEUDORABIES (INFECTIOUS BULBAR PARALYSIS, MAD ITCH) A B SABIN, Brit J Exper Path **15** 372, 1935

Pseudorabies bears no immunologic relationship to rabies Pseudorabies virus possesses many properties in common with the virus of herpes simplex but is easily differentiated from it by active and passive immunity tests Certain hyper-immune antiherpes serums protected guinea-pigs from minimal but constantly infective doses of the virus of pseudorabies Four of fourteen herpes-immunized guinea-pigs resisted a small but definitely infective dose of pseudorabies virus A potent antipseudorabies serum had no effect on herpes A partial immunologic relationship between pseudorabies and herpes is considered possible A generic relationship is suggested for pseudorabies virus, the B virus and the virus of herpes simplex

FROM THE AUTHOR'S SUMMARY

THE FLOCCULABLE SUBSTANCE OF VACCINIA M H SALAMAN, Brit J Exper Path **15** 381, 1934

Seitz filtrates of suspensions of the crusts from vaccinia rabbit's skin possess some antigenic power This power can be definitely enhanced by adsorption of the antigen on particles of collodion A heat-stable flocculating substance may be prepared from the crusts of vaccinia rabbit's skin similar in properties to that obtained by Wilson Smith from vaccinia rabbit's testicle

FROM THE AUTHOR'S CONCLUSIONS

THE AGGLUTINOGENS OF A STRAIN OF VACCINIA ELEMENTARY BODIES J CRAIGIE and F O WISHART, Brit J Exper Path **15** 390, 1934

Agglutinating serum for the elementary bodies of vaccinia, obtained by vaccination of rabbits with or without further inoculation of elementary bodies of the C L strain of vaccine virus, contains two agglutinins The two corresponding agglutinogens differ in their relative stability to heat and other agents The more labile agglutinin designated L has its agglutinability and ability to absorb agglutinin impaired or destroyed by exposure to a temperature as low as 56 C The agglutinin designated S is stable at temperatures up to 95 C

FROM THE AUTHORS' CONCLUSIONS

SPECIES IMMUNITY TO PNEUMOCOCCUS DAVID HARLEY, Brit J Exper Path
16 14, 1935

A solution of pneumococcus type-specific antigen which has been made alkaline and heated to 37 C and injected into mice produces immunity to the homologous and the heterologous types of living pneumococci alike. This new antigen is believed to be a product of a dissociation of the type-specific antigen by the action of the alkali into an antigenic element and a nonantigenic type-specific material. This free antigenic element is common to the type-specific antigens of all the virulent types of pneumococci, it has been designated "pneumococcic species antigen."

FROM THE AUTHOR'S SUMMARY

THE STREPTOCOCCAL COMPLEMENT-FIXATION REACTION IN RHEUMATIC DISEASES
A BECK and F COSTE, Brit J Exper Path 16 20, 1935

Complement-fixation tests have been made on seventy-nine serums from rheumatic patients and on fifty-three control serums. A number of the former group reacted with streptococcic lipoid antigens and failed to react with lipoid antigens of other bacteria or with tuberculous and syphilitic lipoid antigens. Examinations of the control serums showed that the serums of tuberculous persons and of pregnant women often react with streptococcic lipoid antigens. Therefore in assessing the significance of positive streptococcic reactions tuberculosis and pregnancy must be excluded. The positive reaction with a streptococcic antigen has been observed only in those cases of rheumatic disease in which clinically a connection with streptococcic infection was probable. But even in these cases, and also in cases of acute streptococcic infection (scarlet fever, erysipelas), the percentage of serums with positive reactions is not high (seven of forty-four rheumatic serums). It is believed that the sensitiveness of the test is not yet optimal, and an attempt is being made to increase it. If this can be done it may be possible to determine whether rheumatic disease is of streptococcic or other origin.

FROM THE AUTHORS' SUMMARY

HISTOLOGY OF LOCAL HYPERSENSITIVITY REACTIONS R LAPORTE, Ann Inst Pasteur
53 598, 1934

The tuberculin reaction in guinea-pigs is compared with other hypersensitive reactions in the skin. "The tuberculin reaction is clearly distinguished from the anaphylactic reaction of the skin. In the latter the phenomena are more rapid and more severe, necrosis attacks the tissue (notably the epidermis) and electively attacks the vascular walls resulting in intense hemorrhages, this necrosis of the vascular walls is found in the deep layers of the skin, a characteristic which is not seen in allergic reactions. The marked edema, the relative paucity of inflammatory cells in the skin, the predominance of polymorphonuclears and the accumulation of eosinophils constitute the other principal differential characteristics of anaphylactic reactions." Comparing reactions to other irritating substances with the tuberculin reaction Laporte found many points in common, "nevertheless, it is unequivocal that the tuberculous animal reacts by more precocious and notably more important influx of monocytes."

M S MARSHALL

THE CYTOLYTIC AND TOXIC PROPERTIES OF STAPHYLOCOCCI C GENGOU, Arch
internat de med exper 9 413, 1935

Hemolysins, leukocidins and the necrotizing toxin in staphylococcic filtrates are different manifestations of a single product of bacterial metabolism. Since the filtrability of the toxin changes on cultivation of the bacteria in vitro, chloroform has been used in place of filtration for sterilization of the cultures. The amount of precipitate formed in toxic filtrates on addition of antitoxin is not a true index of the strength of the toxin. The precipitate varies with the kind of medium.

ELIZABETH McBROOM

ALLERGIC REACTIONS OF THE KIDNEY M MASUGI and Y SATO, *Virchows Arch f path Anat* **293** 615, 1934

In a previous contribution the authors described renal glomerular changes following the injection of large doses of antikidney serum into rabbits. They considered the changes identical with those of human diffuse glomerulonephritis. These alterations they held to be allergic. In the present work rabbits were sensitized by repeated subcutaneous, intraperitoneal or intravenous injections of horse serum or egg-white. In one series of animals the kidneys were subjected to microscopic examination at various stages of sensitization. In another series the examination was made after the injection into the renal artery of a final activating dose of the foreign protein. In both series the authors observed glomerular changes which they consider identical with the characteristic lesions of human diffuse glomerulonephritis, namely, plasma stasis, fibrin thrombosis and blood stasis in the glomerular vessels. The process involved entire glomerular tufts and the glomeruli throughout the kidney. They conclude that human diffuse glomerulonephritis is an allergic reaction in a hyperergic kidney. In some animals they observed perivascular lesions similar to those of periarteritis nodosa, which, therefore, they also look on as an allergic reaction.

O T SCHULTZ

THE ANAPHYLACTIC REACTION AFTER REMOVAL OF INHIBITORY SUBSTANCES
E BERGER and W MUTSAARS, *Ztschr f Immunitätsforsch u exper Therap* **83** 1, 1934

The addition of an alcoholic solution of cholesterol to the Ringer solution in which the uterine horn of a passively sensitized guinea-pig was suspended inhibited regularly the specific anaphylactic contraction following the addition of the antigen. The mere replacement of the alcoholic solution with fresh Ringer solution brought about a typical contraction. The alcohol was responsible for the inhibition, and not the cholesterol. The effect of histamine was frequently inhibited by the alcoholic solution, but replacement of the latter by Ringer solution was only exceptionally followed by a contraction.

I DAVIDSOHN

THE SPECIFICITY OF ANAPHYLACTIC VASCULAR CONTRACTILITY OF WHITE RATS
K M DWOILAZKAJA-BARYSCHEWA, *Ztschr f Immunitätsforsch u exper Therap* **83** 31, 1934

Segments of arteries were perfused with fluid with and without antigenic substances, and the contraction was measured by the decrease in the number of drops which left the blood vessels. A marked contractility was observed in sensitized white rats, but it was not specific, because heterologous as well as homologous antigens (serums and erythrocytes) were able to produce it. This corroborates the claims of Kritschewski and others that the reaction of the sensitized blood vessels following perfusion of antigens is not identical with the anaphylactic shock.

I DAVIDSOHN

DIPHTHERIA ANTITOXIN IN THE BLOOD OF THE POPULATION IN A TOWN OF JAVA
JEANNE VAN DEN HOVEN VAN GENDEREN, *Ztschr f Immunitätsforsch u exper Therap* **83** 42, 1934

The blood of 1,641 men, women and children of Bandoeng, Java, was studied by means of Romer's technic. A comparative study of the blood of the umbilical cord and of the venous blood of parturient women confirmed the known fact that the amount of diphtheria antitoxin is about equal in both. The native population and the Chinese adults and children had high titers of antitoxin, somewhat lower in women and girls. The titers were much higher than among the European and mixed population, among whom the women showed a particularly low titer.

I DAVIDSOHN

Tumors

BRONCHIOGENIC CANCER COMBINED WITH TUBERCULOSIS OF THE LUNGS B M FRIED, *Am J Cancer* **23** 247, 1935

From a study of thirteen cases of primary carcinoma of the lung combined with pulmonary tuberculosis it was found that the carcinoma had developed independently from the tuberculous disease in some cases, while in others it was engrafted on an old fibrotic infection. The carcinoma did not originate in the wall of the tuberculous cavity but in the basal cells of the adjacent bronchus from where it reached the cavity, lining its wall in a syncytial manner and growing in masses. "Cancerization" of a cavity is analogous to the so-called "epithelialization" of a cavity observed in cases in which the infection with Koch's bacillus shows a tendency toward healing.

In five of the cases the carcinoma was of the small cell variety, in six, squamous epithelial, in one, keratinizing epidermoid, and in one, adenocarcinoma.

All the patients were men. Three showed tubercle bacilli in the sputum, which is contrary to the general belief that the sputum of patients in whom these two diseases are combined is invariably negative for Koch's bacilli.

The tuberculous lesion found in the lungs of the patients was of the healing fibrotic type and was to all appearances of long standing.

The clinical histories and the necropsy observations of the thirteen patients are given in detail. The article is illustrated with ten photomicrographs and ten roentgenograms.

MYXOSARCOMA A A THIBAUDEAU and L C KRESS, *Am J Cancer* **23** 267, 1935

Myxomatous tissue in a tumor is not an evidence of degeneration. Myxomatous tissue is a derivative of connective tissue and can probably originate from the metaplasia of different types of connective tissue. The more malignant a myxomatous tumor, the more cellular it becomes. Myxosarcoma is clinically highly malignant, responding only occasionally to recognized forms of therapy. In general myxosarcoma is resistant to radiation therapy, in some instances, however, it has responded admirably to this type of treatment. Myxosarcoma is most apt to arise between the ages of 40 and 60 years, it shows no special predilection for either sex. The shorter the clinical duration of the tumor before treatment, the more satisfactory is the response to therapy. Myxosarcoma in cases in which recurrences have occurred does not respond to treatment. Widely disseminated metastases occur late in myxosarcoma. Myxofibroma is readily eradicated by appropriate therapy.

FROM THE AUTHORS' SUMMARY

TUMOR TRANSPLANTS FROM MICE TO SPLENECTOMIZED RATS J HEIMAN, *Am J Cancer* **23** 282, 1935

Transplants of mouse carcinoma 63 and 11 and of sarcoma 180 and 37 will not grow in adult splenectomized or in adult normal rats. Mouse sarcoma 37 will grow for a short time in young normal and young splenectomized rats, but recession and absorption of the tumor occur promptly, without visibly affecting the animals. The growth energy of a tumor may be diminished during its stay in foreign soil but is regained when the tumor is transferred to its normal habitat. The presence or absence of the spleen in a mouse or a rat does not seem to hinder or accelerate the growth of sarcoma 37. The age of the animal influences the growth of heterotransplants.

EFFECT OF BROMCAPROIC ACID ON RAT SARCOMA 39 W A SELLE and M BODANSKY, *Am J Cancer* **23** 289, 1935

Bromcaproic acid, despite the fact that it permeates cell membranes more readily than the halogen derivatives of the lower fatty acids, was found to exert

no specific inhibitory effect on the growth of rat sarcoma 39 though given in daily doses equal to one third of the lethal dose over a period of three or four weeks

FROM THE AUTHORS' CONCLUSION

THE INFLUENCE OF MAGNESIUM ON THE GROWTH OF CARCINOMA, SARCOMA AND MELANOMA K SUGIURA and S R BENEDICT, *Am J Cancer* **23** 300, 1935

Transplants of the Flexner-Jobling rat carcinoma in rats fed a diet containing magnesium in the concentration of only 18 parts per million, but otherwise adequate, survived more frequently but grew very much more slowly than such transplants in animals receiving a magnesium-normal diet or fed the common diet. At the end of the third week, the weight of the tumor in the rat fed the magnesium-low diet was only about 4 per cent of that in the animal fed the magnesium-normal diet. The rate of tumor growth in the animals to the diet of which a small amount of magnesium in the form of magnesium sulphate had been added (0.0108 per cent magnesium) was decidedly diminished, but this inhibition was not so great as in the animals fed a diet practically free from magnesium. The resistance of the latter animals to tumor regression was definitely increased over that of normally fed animals. When young rats in which cancerous grafts had become well established were placed on a magnesium-low diet (0.00018 per cent magnesium), there was no marked inhibitory effect on the subsequent rate of tumor growth. When magnesium in the form of magnesium sulphate was restored in adequate amount (0.0538 per cent magnesium) to the magnesium-low diet (0.00018 per cent magnesium) of tumor-bearing animals the tumor nodules grew very rapidly and attained normal size in seven days. A magnesium-high diet (0.1775 per cent magnesium) had a slight but distinct accelerating effect on the growth of the Flexner-Jobling rat carcinoma. However, there was a slight increase in the number of tumor regressions in rats on this diet. Prolonged feeding of the magnesium-high diet did not have any effect in checking the growth of firmly established Flexner-Jobling rat carcinoma, mouse sarcoma 180 and Passey mouse melanoma. The results cast doubt on the value of magnesium in the treatment of human neoplasms. They also show that a neoplasm, like normal tissue, requires a definite amount of magnesium for its growth.

FROM THE AUTHORS' SUMMARY

ON THE PHYSIOLOGICAL VALIDITY OF ENZYME (AMYLASE) DETERMINATIONS IN TUMOR TISSUE F H SCHARLES, P D ROBB and W T SALTER, *Am J Cancer* **23** 322, 1935

A method has been previously described whereby the amylolytic effect of a tumor extract can be expressed as a logarithmic function of the concentration of the extract. Assays are presented of mixtures of varying proportions of two sarcoma extracts. In each case the value obtained agrees with the sum of the theoretically calculated partial activities of the respective components. Because the partial activities are directly additive, it is suggested that the method determines purely the activity of enzyme, and not the effect of accompanying accelerators or inhibitors. This fact substantiates the contention that appropriate determinations of the enzyme activities in tissues have a fundamental physiologic meaning.

FROM THE AUTHORS' SUMMARY

NEUROCYTOMA DERIVED FROM A GANGLIONEUROMA OF THE HYPOGASTRIC PLEXUS SAMUEL J HOFFMAN, *Am J Dis Child* **49** 135, 1935

This case is interesting because it shows the nonmalignant form of neurocytoma in the form of a ganglioneuroma and also the true neurocytoma in the same section. It shows an unusual form of metastasis to many bones in which the periosteum was uniformly thickened.

FROM THE AUTHOR'S CONCLUSIONS

A MALIGNANT HEMANGIOMA OF THE LUNG WITH MULTIPLE METASTASES E M HALL, *Am J Path* **11** 343, 1935

A case of malignant metastasizing hemangioma, of which there are less than a dozen true cases recorded in the whole of medical literature, is reported. The largest tumor was found in the right lung, and death was due to hemorrhage into the right pleural cavity. True metastases consisting of both cavernous and malignant cellular areas were found in the lungs, pleurae, retroperitoneal lymph nodes and liver. The type cell was the endothelial cell, which formed blood-vascular spaces in all the tumor nodules. The cells varied from practically normal-appearing ones lining the cavernous spaces to extremely large atypical cells that almost filled the blood spaces in the more cellular areas. In the latter the growth was rapid and apparently highly malignant.

FROM THE AUTHOR'S SUMMARY

A GANGLIONEUROMA IN THE NECK OF A CHILD J MACFARLAND and S W SAPPINGTON, *Am J Path* **11** 429, 1935

The case described is that of a well characterized ganglioneuroma. In this tumor, however, nerve cells of all stages of development from neuroblasts to ganglion cells occurred, and among them was a stroma made up of Schwann cells and nerve fibers. It occurred in the neck of a little girl, and the case seems to be the twelfth of its kind to be placed on record. Three years after operative removal the patient is living, with no return of the tumor and no metastases. Appended to the article are 143 references to the literature.

FROM THE AUTHORS' SUMMARY

PRIMARY CARCINOMA OF THE LUNG K B OLSON, *Am J Path* **11** 449, 1935

Sixty-nine cases of primary carcinoma of the lung, verified at autopsy, have been presented and divided into three groups: (a) squamous cell carcinoma, (b) adenocarcinoma and (c) undifferentiated carcinoma.

Squamous cell carcinoma constituted the largest single group and 42 per cent of the entire series. The left lung and upper lobes were the most common site of the primary tumor, and in 61 per cent of the cases it involved a bronchus. Cavitation in the primary tumor occurred in 17 per cent. Metastases and extensions were not so widespread as in the undifferentiated group but were more extensive than in that of adenocarcinoma.

Adenocarcinoma constituted 24 per cent of the series and was mucinous in 53 per cent of the cases and nonmucinous in 47 per cent. In all cases the tumor probably originated from the epithelium lining a bronchus or from a peribronchial mucous gland. The mucinous type frequently metastasized and occasionally extended but appeared less malignant than the nonmucinous. It involved bone more frequently than any other type. The nonmucinous type was the least malignant and was occasionally confined to a lobe or a lung. It frequently involved the pleura.

Undifferentiated carcinoma constituted 33 per cent of this series. The primary tumor occurred slightly more frequently in the left lung, always involved a bronchus and occasionally infiltrated an entire lung. It showed the most vigorous tendency to metastasize widely and to extend locally. All the cases occurred in the left lung, in the upper lobes and at the hilus. The primary tumor was a single mass in 95.7 per cent of the cases and usually involved or occluded a bronchus. This type of carcinoma metastasized widely, and the primary tumor was very prone to extend regionally. Skeletal and intracranial metastases were common.

An absolute increase in the general incidence of carcinoma of the lung occurred at the Boston City Hospital in the period from 1930 to Aug. 1, 1934, and is possibly explainable as a selective phenomenon. Males were affected predominantly, in the ratio of 4.5:1. The incidence in males has increased in the past fifteen years. The majority of cases occurred in the sixth and seventh decades of life. Adenocarcinoma tended to occur more frequently at the extremes of life. Asso-

ciated pulmonary inflammatory conditions occurred in 58.8 per cent of the cases. The incidence of pulmonary tuberculosis and pneumoconiosis in this series was consistent with the incidence in unselected cases.

ADENO-ACANTHOMA OF THE PYLORUS J. G. PASTERNAK, *Am J Path* **11** 541, 1935

A case of cornifying epidermoid carcinoma occurring with an adenocarcinoma of the pylorus in which definite transitions from glandular to epidermoid carcinoma were present is reported. The tumor removed at operation was predominantly epidermoid and was confined to the pylorus. No metastases or other tumor foci were demonstrable. At autopsy, the esophagus and cardia were normal, the tumor in the vicinity of the gastric resection was predominantly adenocarcinomatous, and the omentum, lymph nodes and pancreas were infiltrated only by adenocarcinoma.

FROM THE AUTHOR'S SUMMARY

ERYTHROBLASTOSIS G. W. COVEY, *Am J Path* **11** 551, 1935

A brief review of the pertinent literature is given with special reference to the relation between erythroblastosis, erythroleukoblastosis and fetal leukemia. Attention is drawn to the fetal mechanism apparently designed to meet oxygen-poor conditions of intra-uterine life, an erythremia with a large number of immature red blood cells, and to their rapid reduction in number immediately following birth. A possible analogy is pointed out between erythroblastosis and fetal leukemia, on one hand, and adult polycythemia vera and leukemia on the other. The probable role of fetal hydrops and icterus gravis neonatorum as complications of erythroblastosis in a new-born infant having an erythroblastoma in the left pleural cavity is reported, with the observations at autopsy and a description of the histopathologic changes.

FROM THE AUTHOR'S SUMMARY

THE EFFECTS OF GAMMA RAYS OF RADIUM AND OF ROENTGEN RAYS ON LYMPHOMATOSIS OF MICE J. FURTH and D. H. KABAKJIAN, *Am J Roentgenol* **32** 227 and 377, 1934

Continuous exposure of mice with transmitted lymphomatosis to the gamma rays of radium does not prevent the fatal termination of the disease but often prolongs life. Malignant lymphocytes introduced into healthy mice continue to grow under constant exposure to quantities of radiation that cause fatal damage to the host. The gamma rays of radium increase the susceptibility of mice to transmitted lymphomatosis.

Total irradiation of lymphomatous mice by roentgen rays prolonged their life. This effect was proportional to the quantity of roentgen rays applied. Fractional irradiation also prolonged the life of leukemic mice, but under the conditions prevailing in these experiments it was not more effective than single irradiation with a correspondingly large dose. The prolongation of life of leukemic mice by roentgen rays is due to a direct effect of the roentgen rays on the malignant lymphocytes. The death rate from intercurrent diseases was greater among the irradiated than among the nonirradiated animals.

FROM AUTHORS' CONCLUSIONS

BIOPSY IN BONE SARCOMA JAMES EWING, *Am J Surg* **27** 26, 1935

After discussing the objections to typical biopsy in bone sarcoma and its shortcomings, Ewing states that biopsy of material obtained by aspiration with an 18 bore needle has been remarkably successful in revealing the structure of bone tumors. By passing the needle through a minute incision in the skin, after the application of a drop of procaine hydrochloride, it is usually easy to penetrate the shell of a medullary tumor and secure by suction enough tissue to yield a

positive diagnosis With a soft extramedullary tumor the method is practically always satisfactory When the operator acquires some skill, and the pathologist is willing to employ care and patience, a very high proportion of correct diagnoses may be obtained Most of the objections to the surgical biopsy are avoided When this method becomes a familiar routine there remains only a very restricted field for the surgical biopsy

STRUCTURE AND BEHAVIOUR OF THE CELLS IN TISSUE CULTURES OF TUMOURS
R J LUDFORD, *Scient Rep Invest Imp Cancer Research Fund* **11** 147, 1934

Malignant cells of different strains vary in size, in structure, in degree of differentiation and in their manner of growth in mediums which support the growth of normal cells They are less actively motile than polyblasts Their cytoplasm is more finely granular than that of normal cells, their nuclei relatively larger, and the mitochondria usually smaller Abnormalities of mitosis are common They are not stained vitally with neutral red so intensely as polyblasts Most malignant cells fail to stain vitally with trypan blue, like their normal prototypes The rate of growth of malignant cells of the various strains of transplantable tumors in vitro is correlated with their rate of growth in vivo Malignant cells retain the same cytologic features in vitro as in vivo Significant differences occur in the growths of different strains of mouse tumors in mouse and rat serum Explants of some strains grow as large sheets of malignant cells in both serums, others will not in either medium, while some give excellent sheet growths in mouse serum but not in rat serum

THE REACTION OF NORMAL AND MALIGNANT CELLS TO FAT-SOLUBLE COLOURED COMPOUNDS WHICH ARE INSOLUBLE IN WATER R J LUDFORD, *Scient Rep Invest Imp Cancer Research Fund* **11** 169, 1934

Relatively stable colloidal solutions of the fat-soluble, water-soluble colored compounds sudan III and sudan black have been made in serum Serum colored in this manner, sometimes with trypan blue added, has been applied to tissue cultures of normal and malignant cells Fat droplets in both normal and malignant living cells are colored specifically—a vital histochemical reaction Fat droplets in dead and fixed cells stain in the same manner with the colored serum The application of a mixture of sudan III and trypan blue in serum to tumor cultures (Crocker sarcoma, mouse carcinoma 206) results in the fat droplets being stained in both the normal and the malignant cells, but in these experiments only the polyblasts have segregated trypan blue Fat droplets in fibroblasts are colored by sudan III, and small, faintly colored droplets are present in cultures of embryonic fibroblasts to which sudan black has been added Vitally stained fat droplets occur in dividing cells Flocculated and precipitated particles of sudan III and sudan black are segregated, and also phagocytosed, by polyblasts These results suggest that, although malignant cells are readily permeable to fat-soluble substances, they are less permeable to water-soluble compounds than are normal cells The tentative explanation is put forward that the plasma membrane of malignant cells is relatively rich in fatty substances

FROM THE AUTHOR'S SUMMARY

BILE PIGMENT FORMATION IN THE METASTASES OF A CARCINOMA OF THE LIVER
N TOKOZAWA, *Centralbl f allg Path u path Anat* **61** 3, 1934

Evidence is adduced from the following case to indicate the site of the transformation of hemoglobin into bile A laborer, 45 years old, a moderate consumer of alcoholic drinks, noticed distention of and pressure in the upper part of his abdomen and within a month became bed-ridden Repeated paracenteses yielded bloody ascitic fluid Intermittently a subicteric tinge was noted in the ocular conjunctiva, but the skin remained free from icterus, and the bilirubin reaction of the urine was constantly negative Death occurred about six months after the onset

of symptoms, and at necropsy there were found emaciation, a hemorrhagic ascites of 10,000 cc, a primary carcinoma of the quadrate lobe of the liver, the size of a hen's egg, many dark green cancer metastases of the great omentum and visceral peritoneum, and cirrhosis of the liver. The evolution of the primary growth could be traced through cirrhosis to nodular hyperplasia and adenoma formation to carcinoma. Bilirubin was found in abundance in the reticulo-endothelial cells of the metastases. In contrast to this it could be demonstrated only feebly in the tumor cells. The possibility that the bilirubin in the reticulo-endothelial cells occurred as a matter of storage is discredited because there was no dilation of bile capillaries in the tumor or in the cirrhotic liver and no bile cylinders in the gall capillaries to indicate bile stasis. There was, also, no clinical evidence of icterus except occasionally in the bulbar conjunctiva. The blood serum at no time contained quantities of bilirubin indicative of icterus, and a similar negative condition was found in the urine. The author feels justified in concluding that the bilirubin in the reticulo-endothelial cells of the metastases is made in loco and that this case is a contribution to the anhepatocellular theory (Aschoff) of the formation of bilirubin.

GEORGE RUKSTINAT

ASSIMILATORY GROWTH OF CARCINOMA W. SCHILLER, *Virchows Arch f path Anat* **292** 577, 1934

The normal course and arrangement of the epithelial fibrils from the normal to the carcinomatous epithelium observed at the advancing margin of squamous cell carcinomas of the vulva and cervix uteri are interpreted by Schiller as evidence of the transformation of normal epithelium into carcinomatous cells. For such an increase in the size of a carcinoma he prefers the term "assimilatory growth" to "appositional growth."

O. T. SCHULTZ

TUMORS OF THE RETE OVARIUM J. WALLART and S. SCHEIDEGGER, *Virchows Arch f path Anat* **292** 643, 1934

The authors believe that the rete ovarii is derived from the portion of the wolffian duct that gives rise to the primitive kidney. They believe also that the structure is not a useless embryonic rest, but a structure that functions actively in the sexual activities of woman, although the nature of its function is unknown. They describe an ovary removed from a 46 year old woman. The rete had undergone adenomatous proliferation, all transitions from normal to adenomatous structures being evident. In some of the branching tubular structures the epithelium was multilayered, but in general it consisted of a single layer of very tall columnar cells. The epithelium formed papillary ingrowths into the lumen of some of the spaces. In a second case, one of primary bilateral carcinoma of the ovary with metastasis in a woman aged 61, the origin is also ascribed to the rete, on the basis of the character of the epithelium and of the papillary structures formed by it.

O. T. SCHULTZ

Technical

CONFIRMATORY TEST FOR SYPHILIS OF WITEBSKY G. D'ALESSANDRO and F. SOFIA, *Ztschr f Immunitätsforsch u exper Therap* **83** 478, 1934

Witebsky's confirmatory test (abstr, *ARCH PATH* **18** 749, 1934) is based on a liberation of the antibodies from the precipitate which develops in a flocculation test for syphilis. The solution containing the liberated antibodies, which is almost entirely free from protein, is then used for a complement-fixation test. The use of a 1:1,000 dilution of solution of formaldehyde in a physiologic solution of sodium chloride eliminated certain technical difficulties of the original procedure. The test eliminated some of the false positive reactions. In a few cases of mixed syphilitic and gonorrheal infection and of mixed syphilitic and tuberculous infection

the serum from which the syphilitic antibodies were removed fixed complement specifically with the homologous gonorrheal or tuberculous antigen. False positive reactions with serums of patients with malaria could not be eliminated by means of the test. However, a differentiation was possible when an alcoholic extract of human red blood cells was used as the antigen. The antibody-containing solution of the confirmatory test reacted more intensely with the red cell antigen in cases of malaria and vice versa in cases of syphilis, while the untreated serum failed to show any differences with the two antigens. The test was positive in those cases of treated syphilis in which the Wassermann reaction with the untreated serum was positive and the flocculation test negative. Addition of normal serum which had been treated with normal hydrochloric acid changed the solution containing syphilitic antibodies in such a manner that it reacted positively in the precipitation test but negatively in the complement-fixation test.

I DAVIDSOHN

THE CITOCHOL FLOCCULATION TEST FOR SYPHILIS. R. SCHMIEMANN, *Ztschr f Immunitätsforsch u exper Therap* 84 64, 1934

The new modification of the Sachs-Witebsky citochol test which employs 0.1 cc of serum and 0.1 cc of a 1:12 dilution of a 3 per cent solution of sodium chloride proved more sensitive than the old technic which used 0.025 cc of extract in a dilution of 1:3 with physiologic solution of sodium chloride. Schmiemann found that the new technic permits the use of one-half the amount of serum and that it could be successfully adapted for a microscopic slide technic, though there is hardly a need for it. By the use of proper dilutions of serum, the citochol reaction can be well employed as a quantitative test.

I DAVIDSOHN

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

May 13, 1935

I PILOT, M D, *Presiding*

EDWIN F HIRSCH, M D, *Secretary*

A CLINICAL AND EXPERIMENTAL STUDY OF THE MACROPHAGE SYSTEM OF THE LUNGS IN RELATION TO RECOVERY FROM LOBAR PNEUMONIA O H ROBERTSON and LOWELL T COGGESHALL

In an earlier study of the pathology of experimental pneumococcic lobar pneumonia in the dog certain striking histologic changes were observed constantly in the involved lung at the time of recovery, the nature of which suggested the occurrence of local or tissue immunity. In order to determine whether similar changes took place in the human lung in lobar pneumonia a postmortem study was made of the tissues of forty patients dying at intervals of from six to twelve hours to two months after the onset of the disease in whom the approximate age of the different lobar lesions had been determined by x-ray pictures made daily during life.

Histologic changes analogous to those seen in the dog's lung were found wherever resolution was taking place. Often the evolution of the whole process could be followed in a single case in which lesions of different ages were present. The first evidence of reaction consists of an increase in the number of large mononuclear cells in the alveolar walls, many of which protrude into the air spaces. This results in a thickening of the septums. As the process develops the large mononuclear cells become detached from the alveolar wall and enter the exudate where they exhibit the form and phagocytic functions of the macrophages. The latter cells gradually replace the polymorphonuclears, the fibrin disappears progressively and the lesion assumes the characteristic appearance of resolution. The same type of tissue cell reaction was observed in the lymph glands at the hilus of the lung.

Sections obtained from six patients who died at intervals of from six days to two months following recovery from lobar pneumonia showed a pronounced macrophage reaction.

The most striking observation in the study of these human tissues was that wherever a well developed macrophage reaction occurred pneumococci were few or absent while in most lesions of all ages in which the exudate was predominantly polymorphonuclear leukocytes micro-organisms were abundant. Such marked differences in numbers of pneumococci were observed not only between lobar lesions but also at times in different parts of the same lesion where focal macrophage reactions were occurring. The macrophages were seen to be actually phagocytic and showed evidence of effective digestion of the engulfed pneumococci.

Further data on the significance of this reaction were obtained experimentally from certain animals in which clearing of one part of the pneumonic lesion occurred while spreading took place in another part of the lung. The lungs from such animals put to death during the active phase of the disease yielded sterile cultures from the clearing lesion which showed a marked macrophage reaction, while the new lesions contained many pneumococci and exhibited the histologic appearance of a spreading process. Other experimental evidence was presented to indicate that the macrophage reaction represents an immune response.

DISCUSSION

P R CANNON What is the nature of the mononuclear phagocytes?

S C PEACOCK Are patients with chronic bronchitis or other chronic infection of the lungs less susceptible to lobar pneumonia?

O SAPHIR May the presence of macrophages in the gray stage of lobar pneumonia result from organization changes?

O H ROBERTSON The phagocytes have been vitally stained, and most of the cells in the gray stage of pneumonia are macrophages. Patients with chronic bronchitis infrequently contract lobar pneumonia. This may have some relation to the presence of macrophages in these chronic infections. Much evidence indicates that macrophages are concerned with resolution rather than organization. In the latter, fibroblasts are present. The resolving process was studied thoroughly in dogs examined in various stages of lobar pneumonia.

EFFECT OF DISSOCIATION OF STREPTOCOCCI ON THEIR FIBRINOLYTIC AND ANTI-FIBRINOGENIC ACTIVITY RUTH TUNNICLIFF

Tillett and Garner (*J Exper Med* 58 485, 1933) have made the interesting observation that broth cultures of the beta hemolytic streptococcus of human origin rapidly dissolve the normal human fibrin clot. With the exception of some strains of *Staphylococcus aureus* other bacteria, including *Streptococcus viridans*, the pneumococcus and most hemolytic streptococcus strains of animal origin, have not been demonstrated to have this property of fibrinolysis.

Hadfield, Magee and Perry (*Lancet* 1 834, 1934) found greater variation in the dissolving power of their strains of the hemolytic streptococcus than has been described by others. They observed that strains virulent for mice, which formed colonies corresponding to those called smooth in this country, had the strongest fibrinolytic property. As their strains became less virulent they became less fibrinolytic.

For my tests, strains of the hemolytic streptococcus were grown twenty hours in meat extract 1 per cent dextrose broth, p_H 7. If they would not grow in this medium, 1 drop of defibrinated sheep blood was added to 5 cc of dextrose broth.

The method of Tillett and Garner was followed. Since small amounts of blood were more convenient the blood was collected from the finger or ear into a capillary pipet containing 2 per cent sodium citrate in salt solution. Two tenths per cent potassium oxalate gave the same results. Two parts of blood were drawn into a pipet containing 1 part of the anticoagulant and the mixture diluted at once with 8 parts of the physiologic solution of sodium chloride and centrifuged. Four parts of the diluted plasma were then mixed with 2 parts of broth culture and 1 part of 0.25 per cent calcium chloride solution in small test tubes. The mixtures were incubated at 36-37 C. The control containing plasma, un-inoculated broth and calcium chloride clotted in from five to fifteen minutes. When the broth cultures dissolved the plasma clot this occurred in from fifteen minutes to twenty-four hours after its coagulation.

Seventeen hemolytic streptococcus strains from scarlet fever, erysipelas, septic sore throat, malignant endocarditis, septicemia and sore throat were examined for their dissolving activity on normal human plasma clot. All dissolved the clot. I find that the strongest fibrinolytic activity is associated with the virulent strains which have capsules and produce smooth colonies. This property is gradually lost as the cocci form more granular colonies and finally colonies that are dull, rough and flat with irregular, lacy edges, and as they change from round diplococcal forms into the large round or flattened cocci in twisted chains or into the bacillary and filamentous forms characteristic of the rough type of streptococcus. They generally lose their dissolving power before they are completely stabilized as rough. As the rough forms revert to the smooth, morphologically and colonially, the cocci again acquire the power to dissolve the fibrin clot.

My observations are in accord with those of Dennis and Berberian (*J Exper Med* 60 581, 1934) that strains of *Str viridans* do not dissolve the fibrin clot but may inhibit its formation. This property seems to be associated with the smooth type of colony. I examined five strains from subacute and malignant endocarditis, two from measles and one from sore throat. One strain of a non-hemolytic streptococcus from endocarditis behaved like the greening cocci. Like the hemolytic streptococcus, the green-producing streptococcus associates virulence with the smooth type of colony. As the cocci of smooth strains change into those of the rough type they lose virulence and show the same morphologic and colonial variations as are seen in rough cultures of the hemolytic streptococcus. When they revert to the smooth form of colony they also revert morphologically. I find that the anticlotting property of *Str viridans* is associated with the smooth virulent type of colony and as the cocci change into the rough form they lose this property, but reacquire it as they revert to the smooth type.

Although the greening colonies may appear smooth for several days, if the broth culture does not prevent clotting of the plasma rough projections with their bacillary forms will be seen at the edge of the colony, generally within a week.

To determine whether a streptococcus broth culture is of the smooth or of the rough type it is advisable to plate onto blood agar and examine with a culture microscope for rough colonies.

Summary—The fibrinolytic activity of the hemolytic streptococcus appears to be associated with the virulent smooth type of colony. The power is lost as the colony becomes rough, and is regained as it reverts to the smooth form.

Str viridans does not have power to dissolve the fibrin clot but may prevent its forming. This antifibrinogenic activity is associated with the virulent smooth type of colony, is lost as the colony changes to the rough avirulent form, and is regained as it reverts to the smooth type.

DISCUSSION

O. H. ROBERTSON: Have you studied the fibrinolytic and antifibrinogenic properties of various immune serums?

R. TUNNICLIFF: I have made no such studies.

RENAL DENERVATION. EFFECT OF DAILY INJECTIONS OF COION BACILLI AND PITRESSIN ON THE DENERVATED KIDNEY OF THE DOG. GEORGE MILLES and MAURICE HARDGROVE.

The article appeared in full in the October 1935 issue of the ARCHIVES, page 548.

DISCUSSION

H. JAFFE: These experiments should be tried on animals that are not so prone to spontaneous fibrosis of the kidneys.

G. MILLES: We consider the experiments adequately controlled even though dogs only were used.

CYANOTIC ATROPHY OF THE LIVER. A WAX MODEL RECONSTRUCTION. C. S. HAGERTY and J. W. DEVEREUX.

This article will be published in full in the ARCHIVES OF PATHOLOGY.

SUPPURATIVE AORTITIS. REPORT OF TWO CASES. MARSHALL Q. BAKER.

Two cases of suppurative aortitis are described, both associated with pre-existing syphilitic aortitis.

Rupture of the supra-auricular portion of the aorta with hemopericardium occurred in one. The causative micro-organisms (pneumococci and streptococci) were demonstrated and the probable pathogenesis was indicated in each one.

The condition has never been diagnosed clinically, always being discovered incidentally post mortem.

BUFFALO PATHOLOGICAL SOCIETY

*Regular Meeting, May 24, 1935*KORNEL TERPLAN, *President, in the Chair*W F JACOBS, *Secretary*CARCINOMATOUS CIRRHOSIS OF THE LIVER WITH SARCOMATOSIS OF THE PERITONEUM
S SANES and E F COOK

A case is reported because of the occurrence of two different types of malignant neoplasm with typical atrophic cirrhosis of the liver. That a pathogenic relationship exists between Laennec's cirrhosis and primary carcinoma of the liver is generally recognized. Whether the association of an endothelial sarcoma of the peritoneum with the cirrhosis in this case was more than a coincidence seemed an interesting point for discussion.

An Italian, 57 years of age, was admitted to the hospital on Nov. 29, 1934. All his adult life he had partaken of wine and whiskey daily. He first began to lose weight and strength in 1932. In March 1934 he complained of cramplike abdominal pain, diarrhea and bloating. The liver and spleen were distinctly palpable, the legs were edematous. Fluid was demonstrated in the abdominal cavity. Urobilinogen was present in the urine, the van den Bergh reaction was positive. In the last six months of the patient's life fluid accumulated rapidly in the abdominal cavity. Samples obtained by paracentesis showed no tumor cells. The patient died rather suddenly.

The anatomic diagnosis was marked Laennec's cirrhosis of the liver with typical small-nodular regeneration, chronic splenic tumor (600 Gm), varices of the lower esophageal veins with recent rupture in one vein, marked gastroenterorrhagia, slight icterus of the skin, multiple carcinomatous nodules in the liver, ranging from walnut size to small peach size and showing distinct necrosis and icterus (cirrhosis carcinomatosa), primary endothelial sarcoma of the omentum, mesentery and entire visceral and parietal peritoneum, hemorrhagic ascites (2,000 cc).

The omentum, mesentery, visceral and parietal peritoneum were studded with tumor nodules ranging from pea size to cherry size. Many were pedunculated and hemorrhagic. Their surfaces were smooth, their consistency, firm, their color, grayish. Several nodules floated in the ascitic fluid. Where the peritoneum presented no nodules it was opaque. No metastases were found in lymph nodes. Histologically the tumor proved to be an endothelial sarcoma. In uninvolved areas of the peritoneum there was a distinct proliferation of serosal cells. They were increased in size and formed several layers. The subserous tissue was distinctly thickened. It contained many dilated lymphatic vessels. A few of these were lined with hyperplastic endothelial cells. The tumor nodules themselves disclosed various stages of growth. In the immature type the cells varied in shape and size. They were predominantly fusiform or polyhedral. Syncytial processes could be seen. Unnucleated and multinucleated giant cells were present in abundance. Silver impregnation stains revealed reticulum fibers in the stroma. The cells in the mature type were practically only fusiform, they appeared to be arranged in bundles. Small capillary vessels were prominent. Invasion of the outer muscle layer of the intestine had taken place.

DISCUSSION

K TERPLAN. Macroscopically this case was first a diagnostic problem. The primary character of the multiple nodular tumors of the mesentery and omentum was readily recognized. The soft tumor nodules in the cirrhotic liver, however, were first thought to be metastases of the peritoneal sarcoma. Although the

combination of these two different malignant blastomas in a case of Laennec's cirrhosis is certainly most unusual and known to few, if known at all, the temptation is great to look for some pathogenic relationship between this primary sarcoma of the peritoneum and chronic ascites from atrophic cirrhosis. In chronic ascites from portal obstruction distinct thickening of the visceral and parietal peritoneum, so-called plastic peritonitis, is usually observed. It is believed that this proliferation of the mesothelial and subserous mesenchymal cells is a resorptive (?) effect of chemical (toxic) irritation rather than of continuous pressure from the large volume of fluid alone. Thus this prolonged irritation of the lining surface of the peritoneal cavity could be considered as *one* factor bringing about, besides mere hyperplastic thickening of the serosal cells, true blastomatous proliferation. A second point of interest is that lymphogenic and hematogenous metastases from the primary peritoneal sarcoma were not discovered.

PATHOLOGY OF YAWS, ESPECIALLY THE RELATION OF YAWS TO SYPHILIS H U WILLIAMS

This article appeared in full in the October 1935 issue of the ARCHIVES, page 596

FAT CONTENT IN THE BLOOD OF THE RIGHT SIDE OF THE HEART IN A CASE OF FATAL FAT EMBOLISM K TERPLAN, R S HUBBARD and C T JAVERT

This case is presented for two reasons. The diagnosis of fat embolism was made on gross inspection of the blood in the right side of the heart, and the fat content of the blood from the right ventricle was quantitatively determined.

A white man, 70 years of age, was injured in an automobile accident. A fever of 101 F developed, the pulse rate was 80, the respirations 18, the blood pressure 62 systolic and 40 diastolic. The fever rose to 105 F, and the patient died about four hours after the accident. The chief observations post mortem were a depressed fracture of the frontal bone, fractures of the seventh cervical and the seventh to tenth thoracic vertebrae, multiple fractures of the right femur and patella, minor contusions and lacerations, subarachnoid hemorrhage and lacerations of the frontal lobe. The right side of the heart and the pulmonary artery contained fluid blood with innumerable fat globules on its surface. The fluid blood in the left ventricle and aorta did not contain visible fat. The foramen ovale was closed. The lungs felt greasy to palpation. Fat was readily demonstrated in the pulmonary capillaries in stained sections but not in the brain or in the blood obtained from the left ventricle.

Blood taken from the right ventricle and pulmonary artery was chemically analyzed. The specimen, measured in a graduated cylinder, consisted of approximately 8 cc of a mixture of gross fat and partially hemolyzed blood. There was no evidence of the presence of an emulsion, as drops of fat were floating on the surface of the liquid.

The material and container were repeatedly extracted with a mixture containing 3 parts of alcohol and 1 part of ether. The extract was evaporated to dryness and the residue taken up with ether. After further purification by repeated evaporation and resolution in ether the extracted lipoids were dried on a water bath and brought to constant weight in a desiccator. The lipid recovered weighed 1.251 Gm.

One and one-tenth grams of the material was found to be in the form of "total fatty acid" (Bloor, W F, Pelkan, E F, and Allen, D M *J Biol Chem* 52 191, 1922). Titration with sodium alcoholate in benzene showed that none of this was in the free form. Also present were 0.67 mg of organic phosphorus (Harnes, A R *J Biol Chem* 77 405, 1928) and 11 mg of cholesterol (Bloor, W R *J Biol Chem* 24 227, 1916). The iodine number (Ralls, J O *J Am Chem Soc* 121, 1934), corrected for the cholesterol, was 55.3. This corresponds to that of a mixture of glycerides of stearic and oleic acid containing 64 per cent triolein.

There was less pigment soluble in petroleum ether than was found in a comparable amount of fat extracted from normal blood. There was, however, a small amount of a pigment present which was not found in fat from normal blood. This pigment was yellow-brown and was easily soluble in ethyl ether but insoluble in petroleum ether.

Blood from the right ventricle of a patient dying from fat embolism contained between 15 and 16 Gm. of fat per hundred cubic centimeters of fluid. The extra lipid was in the form of a neutral fat with a fairly low iodine number.

Obituaries

EDWIN RAYMOND LeCOUNT

1868-1935

Edwin Raymond LeCount was born in Wisconsin on April 1, 1868. He entered Rush Medical College from Carroll College at Waukesha, Wis., in 1889 and received his medical degree in 1892. A year and a half later he completed his internship in the Cook County Hospital.

At that time the instruction in pathology in Chicago was at the best rudimentary and superficial. In the hospitals there were no pathologic examinations with one glorious exception—the demonstrations by Christian Fenger in the necropsy room of the Cook County Hospital and in connection with his surgical clinics. By arousing interest in pathology and in a deeper understanding of disease, this master pathologist profoundly influenced the ideals and careers of many young physicians, including Dr. LeCount. There was then hardly any sustained or systematic medical research of any kind in Chicago, with rare exceptions, the output consisted of reports of clinical observations. But the end of a period in American medicine was at hand, a new era was coming with new facilities and fresh enthusiasm. Putting his trust in the future, Dr. LeCount, soon after his internship, began working in pathology at Rush Medical College, which he was to serve faithfully to the end of his days. Since about 1902 he had charge of the work in pathologic anatomy.

He at once set to work to strengthen his fundamental training. He developed a concise and orderly style of writing. He mastered French and German when he realized his need of direct access to the medical literature in these languages. He spent several months in Dr. Welch's laboratory in the Johns Hopkins Hospital. He acquired and maintained a remarkably good understanding of the chemical, physical and microbiologic factors in pathologic morphology. His two periods of study in Europe were centered, one on microbiology at the Pasteur Institute in Paris in 1896, and the other on chemistry in Berlin and Halle in 1905. He became essentially a pathologic anatomist of the classic type, devoted primarily to teaching and advancing the knowledge of the morphologic aspects and genesis of the structural changes in disease and injury. He accumulated a rich and varied experience in the laboratory as hospital pathologist and as physician to the coroner's office of Cook County for thirteen years (1911 to 1924). He set a high standard for necropsy and for necropsy records. In connection with his medicolegal work, he developed a well organized system of volunteer assistantships. The duties

were indeed arduous, but the opportunities to learn were so attractive that there never was any lack of acceptable applicants. The work began between 4 and 5 o'clock in the morning, so that it might be finished before the hour of the regular course in pathologic anatomy. So tireless was Dr. LeCount's industry, so fully had he taken himself in hand, that during these years he rose shortly before 4 o'clock. In the course of



EDWIN RAYMOND LeCOUNT
1868-1935

his medicolegal service he gathered a remarkable series of records of cases (99 volumes, fully indexed), which form a copious source of information concerning the pathologic anatomy of medicolegal conditions. He was engaged in the study of this material to the last, and in his room in the hospital he completed valuable analyses of cases of fractures of the skull and of gunshot wounds involving the chest and abdomen simultaneously.

As the years passed he developed into an authority in practical pathology, clinical and medicolegal. His experience, the skill and thoroughness of his examinations, his wide knowledge, his refusal to come to any final decision in the absence of conclusive evidence and the soundness of his judgments gave him the standing of a supreme judge in questions of diagnosis on a structural basis.

The plan Dr. LeCount eventually followed in teaching pathologic anatomy was in brief to give each student full opportunity to learn through his own efforts—no so-called spoon feeding. "The materials are before you. What do you see and what does it mean? Here are references to the literature." It was an earnest effort to get the real work of the course done by the student himself. He frequently told his students that he would not "rob them of the joy of discovery." To many students the plan was not immediately acceptable, they expected to receive more direct instruction, to be told more about what they were supposed to discover and to interpret. Many were not satisfied with their own efforts. Perhaps the method was too difficult, at least in some cases. But on the whole a consensus developed, which grew stronger with time, that the method was far more effective than anticipated. "He made me think," "he taught me to see for myself," "he trained me to discover morbid changes independently" are comments that illustrate the feeling of former students as time lengthened their perspective. No higher praise could be given, and the records of his students and assistants leave no doubt of the great value of his teaching. He was tireless in his efforts to arouse in them the spirit of investigation. Indeed, his whole plan of instruction was based on independent effort. Probably not a single class completed his course without one or more members being singled out for special work, usually on remarkable cases or on some concrete problem. The "Transactions of the Chicago Pathological Society" contain many reports of such work. Many clinicians and pathologists, including not a few of both groups who are now in important academic positions, received through him the introductory investigative stimulus. The value of this influence in the promotion of research cannot be overestimated.

Significant of Dr. LeCount's deep concern in research at an early stage in the medical career is his gift in 1931 of \$10,000 to the Institute of Medicine of Chicago "for a trust fund to bear the name of Joseph Almarin Capps, with the provision that the income from the fund be used to establish a prize to be awarded each year for the most meritorious investigation in medicine in Chicago completed within two years after graduation by a graduate of a medical school in Chicago." So far four Capps prizes of \$500 each have been awarded.

Dr. LeCount himself made important contributions to pathology. His studies of the microscopic lesions in Rocky Mountain spotted fever, based

on material furnished him by Howard T. Ricketts, and of the earliest changes in the lungs in influenza are especially noteworthy. He recorded observations of great value on coma, injuries and embolism. In 1934 the medical faculty of the University of Cincinnati bestowed on him the James E. Stacy Award for "his experimental studies on the isolation of streptococci from sore throats and the experimental induction, through their injection, of acute, healing and scarifying types of nephritis, identical with forms of chronic nephritis observed in man."

Dr. LeCount dealt earnestly with facts and situations, and he pursued his objectives in a distinctive and determined way. He was also of a highly sensitive nature and subject to reactions of a defensive tendency, which sometimes may have been misunderstood. In the midst of the day's work he might be blunt and abrupt, but beneath the surface beat a warm and kindly heart. Children loved him instinctively, and he liked old people. He was unassuming, generous, helpful. To his friends he was frank and finely loyal. Sincerity, high standards and an underlying idealism guided his course.

LUDVIG HEKTOEN

Book Reviews

Unfall und Hirngeschwulst Ein Beitrag zur Aetiologie der Hirngeschwulste By Prof Dr Otto Marburg, Vorstand des Neurologischen Institutes der Wiener Universität Price, 8.80 marks Pp 106, with 12 illustrations Vienna Julius Springer, 1934

The question of the relationship of trauma to the subsequent development of neoplasms is one of great interest, particularly to the pathologist and those concerned with the legal aspects of medicine. In this short monograph Marburg is concerned with the relationship between injuries of the head and the development of tumors of the brain. Following a discussion of the neuroglia and its development he presents three cases of his own, one of a medulloblastoma of the cerebellum, one of a "polymorph celled" glioma and a third in which the exact nature of the tumor is not clear. In all three cases the manifestations of the tumor were preceded by an injury to the head.

The author then presents a review of similar cases reported in the literature. In all, he has collected about one hundred and forty-one instances in which some injury preceded the appearance of the cerebral neoplasm. Each case is briefly abstracted. Following a discussion of these cases Marburg considers at some length the pathogenesis of tumors of the brain, particularly gliomas, and the relationship of their development to trauma. His conclusion is that there can be no doubt of the connection between injuries of the head and the development of intracranial neoplasms. The influence of such trauma he believes may be exerted in two ways. Either the trauma may disturb an existing embryonic rest, causing it to undergo neoplastic changes, or the trauma may produce some structural change, a scar or the introduction of a foreign body, which may stimulate the tissues to neoplastic formation.

The presentation of this material, although a valuable expression of opinion from the leading Austrian neuropathologist, should not be accepted without serious consideration. The mere temporal relationship between a given injury and the development of a tumor does not establish the trauma as the causative factor. Such "post hoc" argument is among the most common and dangerous in medicine. Until definite proof, such as that persons who have suffered from severe injuries of the head are more subject to the development of intracranial tumors than other persons, is forthcoming, the thesis that such tumors may develop as a result of trauma must be considered as unproved. At the moment evidence seems to be pointing away from any real causative relationship between trauma and the development of neoplasms.

Die Hormonforschung und ihre Methoden By Max Reiss, Dr med, Dr rer nat, Privatdozent für pathologische Physiologie an der Deutschen Universität in Prag Price, 19 marks Pp 415, with 26 illustrations and 7 charts Berlin Urban & Schwarzenberg, 1934

Reiss, who was associated with Arthur Biedl for a number of years, set out to write a brief and compact book on internal secretion that would include (a) the established results and (b) the methods of physiologic investigation. The book was meant for the research worker. Another aim of the book was to include the methodological experience that accumulated in the course of years in Biedl's institute.

The results of physiologic, pharmacologic and chemical phases of hormonal research are discussed in the first part of the book in 261 pages. The thyroid, the parathyroids, the thymus, the hypophysis, the adrenals, insulin and the ovarian and testicular hormones are treated with a clarity and completeness that are admirably combined with economy of words and space. The book can be highly commended.

for the sobriety of its approach and for its critical analysis of the difficult and, in places, vague subject. Still more valuable than the first and theoretical part is the second part of the book, which in 142 pages treats of the methods used in endocrinologic research. A number of methods are presented for each procedure, with a critical discussion of their merits and shortcomings.

Some readers will welcome the inclusion of earlier methods, not so much for their practical value as for reasons of historical interest. To have so many technical details compiled in a handy volume will prove helpful to many. The style of the author is very clear. Exhaustive bibliographic references and eighteen columns of a subject index are appended. A stimulating two-page discussion of the relation between endocrinologic research and medical practice concludes the book.

Contribution à l'étude de la variabilité du virus tuberculeux By P. Denys
Pp. 90, with 6 illustrations. Louvain: Imprimerie Saint-Alphonse, 1935.

The general conclusions from this thesis are: The variability of a microbial species manifests itself principally in its morphologic, cultural and serologic characteristics, as well as in its pathogenic power. These are the different aspects of the problem of variability which have been studied with reference to the bacillus of tuberculosis. The experiments show that its attributes are highly variable, so that it is possible to transform a typical Koch bacillus to a bacillus which has all the characteristics of a paratuberculous saprophyte. In a study of the variations of BCG it has been found that it is possible to modify its cultural and biochemical characteristics and at the same time to induce it to acquire pathogenic power, no doubt rather low but superior to that of the nonmodified strain. It has not been possible to secure any evidence of a tuberculous ultravirus. This problem requires new investigations in which consideration should be given to recent work on spontaneous infections of guinea-pigs.

Books Received

LABORATORY MANUAL OF THE DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY, PEIPING UNION MEDICAL COLLEGE Prepared under the direction of C E Lim, Head of Department Second edition Price, \$1 50 Pp 190 Peiping, China Kwang Yuan Press, 1935

EAR EXOSTOSES Smithsonian Miscellaneous Collections, Vol 93, No 6, Publ 3296 Aleš Hrdlička, Curator, Division of Physical Anthropology, United States National Museum Price, 50 cents Pp 100, with 5 plates Washington, D C Smithsonian Institution, 1935

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF VISION, XIV CHARACTERISTICS OF DICHROMATIC VISION WITH AN APPENDIX ON ANOMALOUS TRICHROMATIC VISION Medical Research Council, Special Report Series, No 200 F H G Pitt Price, 1s 3d Pp 58 London His Majesty's Stationery Office, 1935

REPERTORIO SISTEMATICO DEI MICETI DELL-UOMO E DEGLI ANIMALI Arturo Nannizzi Price, 100 lire Pp 556 Siena s a poligr Meini, 1934

RONTGENBEFUND UND PATHOLOGISCH-ANATOMISCHER BEFUND BEI LUNGENKRANKHEITEN VERSUCH EINER KRITISCHEN VERGLEICHUNG Dr med Max Verse, o o Professor der allgemeinen Pathologie und pathologischen Anatomie, Direktor des pathologischen Instituts der Universitat Marburg In 2 volumes Price, 18 marks Part 1, text, pp 96, part 2, atlas, with 144 illustrations Berlin Otto Elsner Verlagsgesellschaft, 1935

VORKOMMEN UND VERBREITUNG DER THYREOTOXICOSE IN SCHWEDEN ZUR GEOGRAPHISCHEN PATHOLOGIE DES MORBUS BASEDOWI UND VERWANDTER KRANKHEITEN Thor Sallstrom Pp 296, with 52 figures Stockholm Klara Civiltryckeri A-B, 1935

THE WISTAR INSTITUTE STYLE BRIEF A guide for authors in preparing manuscripts for the most effective and economical method of publishing biological research Prepared by the cooperative efforts of the editors of journals published by the Wistar Institute and the staff of the Wistar Institute Press Price, \$2 Pp 169, with 23 figures and 37 plates Philadelphia The Wistar Institute Press, 1934

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THE PARATHYROID GLANDS

II A HISTOLOGIC STUDY OF PARATHYROID ADENOMA

SHIELDS WARREN, M D

AND

J R E MORGAN, M D *

BOSTON

For the past few years the various phases of the abnormal body states arising as a result of a paucity or an excess of the secretion of the parathyroid glands have intrigued investigators in all divisions of medical science. Many aspects of the problem have been studied and solved by the cooperation of physiologists, biochemists, endocrinologists, roentgenologists and surgeons. There remain, however, the pathologic study and classification of the parathyroid glands from patients who have suffered from an excess or abnormal secretion of these glands. At the time this study was undertaken, no acceptable classification of parathyroid tumors was available, but during the course of our investigations Castleman and Mallory¹ presented their work on this phase of the problem. In this report the workers collected from the literature and summarized 160 cases of disorders that were proved to be or that were probably hyperparathyroidism. Since this summary contains all the important data and is readily available, our work covering these cases has been deleted. To these cases Castleman and Mallory added 25 of their own, making a total of 185. The greatest numbers of cases previously reported were 5 by Hunter and Turnbull² and 6 by Bergstrand³.

HISTORY

The principal points of historical interest concerning the discovery and development of knowledge of the normal gland have been reviewed in another publication⁴. A large number of papers have been written

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From the pathological laboratories of the New England Deaconess Hospital, the New England Baptist Hospital and the Pondville State Hospital

1 Castleman, B, and Mallory, T B. *Am J Path* **11** 1, 1935

2 Hunter, D, and Turnbull, H M. *Brit J Surg* **19** 203, 1931

3 Bergstrand, H. *Acta med Scandinav* **54** 539, 1921, *Endocrinology* **6** 477, 1922, *Acta path et microbiol Scandinav* **5** 52, 1928, *Acta med Scandinav* **76** 128, 1931, *Am J Cancer* **21** 581, 1934

4 Morgan, J R E. *Arch Path*, to be published

on the various aspects of parathyroid disease, and since many excellent historical reviews of the subject are readily available further repetition is unnecessary. It is of interest, however, to note that the first parathyroid tumor was not described until 1900 (de Santi⁵), and it was not until 1925 that Mandl⁶ definitely related hyperparathyroidism to generalized osteitis fibrosa cystica. Among the more comprehensive writings are those of Albright and his co-workers,⁷ Collip,⁸ Hunter and Turnbull,² Beigstian,³ Welti,⁹ Jung,¹⁰ Churchill and Cope,¹¹ Compere¹² and Castleman and Malloy.¹ The recent publication by the latter two investigators contains an excellent review of the literature to date.

REPORT OF CASES

This report presents 6 cases of parathyroid tumor with associated hyperparathyroidism. Five of the cases were in patients of the Lahey Clinic, and in each one Dr. Frank H. Lahey found and removed a parathyroid tumor. The sixth case was discovered only at autopsy, the roentgenologic observations having been misinterpreted owing to an associated carcinoma of the breast.

CASE 1—A white woman, aged 62, entered the New England Deaconess Hospital for treatment of vulvovaginitis. Routine examination revealed marked kyphosis, loss of stature and rarefaction of all bones except those of the skull and feet. The serum calcium was 14 mg per hundred cubic centimeters, the phosphorus, 2 mg, and the phosphatase elevated. The calcium balance was negative. Hyperparathyroidism was diagnosed.

There was no palpable tumor, but an exploratory operation revealed below the lower left lobe of the thyroid a mass which extended down into the anterior part of the mediastinum and was distinctly separated from the thyroid except for blood vessels and areolar tissue. On the third day after operation the serum calcium was 9.8 mg, and mild tetany was present. Later the serum calcium fell to 7.1 mg, and the phosphorus rose to 2.4 mg. With adequate treatment the patient made a good recovery and was discharged four weeks after operation.

5 de Santi. Internat. Centralbl. f. Laryng. u. Rhin. **16** 546, 1900.

6 Mandl, F. Wien klin. Wchnschr. **38** 1434, 1925. Zentralbl. f. Chir. **53** 260, 1926.

7 Albright, F., Bloomberg, E., Castleman, B., and Churchill, E. D. Arch. Int. Med. **54** 315, 1934. Albright, F., Baird, P. C., Cope, O., and Bloomberg, E. Am. J. M. Sc. **187** 49, 1934. Albright, F., Aub, J. C., and Bauer, W. J. A. M. A. **102** 1276, 1934. Albright, F. New England J. Med. **209** 476, 1933. Bauer, W., Albright, F., and Aub, J. C. J. Clin. Investigation **8** 229, 1930.

8 Collip, J. B. J. Biol. Chem. **63** 395, 1925. Medicine **5** 1, 1926. Canad. M. A. J. **24** 646, 1931.

9 Welti, H. Chirurgie des parathyroides, in Rapport, Association française de Chirurgie, XLII. Congrès français de Chirurgie, Paris, Brodard & Taupin, 1933.

10 Jung, A. Chirurgie des parathyroïdes, in Rapport, Association française de Chirurgie, XLII. Congrès français de Chirurgie, Paris, Brodard & Taupin, 1933.

11 Churchill, E. D., and Cope, O. Surg., Gynec. & Obst. **58** 255, 1934.

12 Compere, E. L. Surg., Gynec. & Obst. **50** 783, 1930.

Gross Description—The tumor mass measured 3 by 2 by 1.5 cm., was well encapsulated and of a yellowish-pink mottled color. The tissue was soft and on section bulged slightly above the cut surface. Sections for microscopic study were taken from each end of the tumor, one of which was a deeper yellow than the other.

Microscopic Description of Section A—Section A (fig 1A) consisted almost entirely of transitional oxyphil cells which were slightly larger than normal,



Fig 1 (case 1) —A, parathyroid adenoma, $\times 275$. Note the uniformity of the cells and the irregularity of the fibrous tissue. This is a hematoxylin-eosin preparation. The granules are unstained. B, marked cellular variability, $\times 825$. Note the nuclear gigantism and multiplicity.

varying from 9 to 16 microns in diameter, with the average cell measuring about 12 microns. The cells varied in shape from round to polygonal, with the cell membrane usually distinct. With the modified Mallory stain the cytoplasm

contained a variable amount of reddish granular material. The nuclei were slightly larger than normal and, though chiefly of the dark type, other transitional forms were also present. Scattered throughout the section were a few principal and oxyphil cell forms. The latter cells had an average diameter of about 18 microns and except for a slight increase in size and diminution in intensity of granular staining were similar to the normal oxyphil cell.

The stroma was irregular in amount and distribution, being thick and collagenous in some areas and thin and stringy in others. This fibrous tissue divided the cells into irregular cords and clumps, with a few alveolar structures also present. The acinar walls were composed of transitional oxyphils with an occasional mature oxyphil form and, though many acini were empty, some contained a clear colloid substance. The vascular network showed a moderate increase in amount and an irregularity of vessel caliber and distribution. A few fat cells, singly and in small groups, were also scattered throughout the tissue.

Summary This section of the tumor was composed chiefly of transitional oxyphil forms with a few normal principal and oxyphil cells also present. There was moderate and irregular increase in the vascular supply and fibrous tissue stroma. The cells contained no mitotic figures nor did they present evidence of active proliferation.

Diagnosis Transitional oxyphil cell adenoma

Microscopic Description of Section B—The cells of this section (fig 1B) were so heterogeneous and variable that their description is difficult. The most striking feature was the magnitude of the cells, many of which measured up to 50 microns in diameter, with the average cell diameter being about 25 microns. The amount of cytoplasmic granularity was extremely variable, ranging from a closely packed mass of acidophilic granules to a perfectly clear ballooned cell. The nuclei presented the most distinctive features, being chiefly large, round or oval and taking a pale stain. Most of the nuclear chromatin consisted of finely divided sandlike particles, but a few pyknotic nuclei were also present. The nuclei measured from 6 to 20 microns in diameter with an average of about 12 microns, and each contained from 1 to 4 bright red irregular nuclei which were slightly larger than normal. Many of the cells had multiple nuclei, the number ranging from 2 to 6.

The fibrous tissue stroma presented a slight increase in amount and was irregularly distributed. The cells presented a protean arrangement with no alveolar formation. As in the other section, the vascular supply was increased in amount and irregular in distribution.

Summary The majority of cells most closely resembled the transitional oxyphil type but showed giant forms with nuclear gigantism and multiplicity. There were no mitotic figures, but the multinucleated cells were indicative of amitotic division. There was a slight irregular increase in fibrous tissue and vascular supply.

Though the two sections were taken from the same tumor and classified as being of the same type (transitional oxyphil), there was a marked variation in cell forms. Section B showed a much greater variation in cell types from one area to another and often within the confines of a single low power field.

Diagnosis Transitional oxyphil cell adenoma

CASE 2—A white woman, aged 52, entered the New England Baptist Hospital with the complaint of pain in the knees and neck and partial loss of function of various joints. Examination revealed marked arthritic changes of the cervical vertebrae, decalcification of the femurs and crepitation and thickening of the synovial membrane of the knee joints. The serum calcium was 12.5 mg, and

the phosphorus and phosphatase were within normal limits. The calcium balance was negative. The diagnosis was atrophic arthritis and hyperparathyroidism.

There was no palpable mass, but at operation the right inferior parathyroid was found to be greatly enlarged. On the second day following operation the serum calcium was 10.9 mg and the phosphorus 2.8 mg. The patient did not have tetany and on discharge, ten days after operation, had normal amounts of serum calcium and phosphorus.

Gross Description—The tumor consisted of a rounded, fully encapsulated mass of tissue measuring 1 cm in diameter. It was moderately firm in consistency and of a reddish-brown mottled color. Section revealed a reddish-brown mottled moist surface which bulged slightly.

Microscopic Description—All cells of this tumor corresponded to the types seen in the normal gland. There was, however, a great preponderance of oxyphilic forms, both transitional and mature, with comparatively few principal cells. Except for an occasional *wasschelle* form, all cells contained red granules which stained slightly lighter than normal. Virtually every cell was larger than its normal prototype, with many of the oxyphil forms measuring up to 30 microns in diameter. The nuclei were for the most part large, round and swollen, with a light staining reaction, and from 1 to 3 prominent, deeply acidophilic nucleoli. An occasional oxyphil form was present which contained 2 or 3 large pale nuclei, but no mitotic figures were seen.

The fibrous tissue stroma was in some areas irregularly thickened and in others stringy and less abundant. This tissue divided the cells into irregular clumps and masses with no definite arrangement or alveolar structures present. The sinusoidal capillaries showed a marked variation in caliber and regularity of distribution.

Summary Most cells were of the transitional oxyphil type with a slight but definite increase in size. Cellular gigantism and nuclear multiplicity were present but not frequent. There was an irregular increase in fibrous tissue and vascular supply. No mitotic division was demonstrable.

Diagnosis Transitional oxyphil cell adenoma.

CASE 3—A white woman, aged 48, entered the New England Baptist Hospital with a palpable adenomatous goiter and a complaint of nervousness, pain in the legs and a loss of 40 pounds (18.1 Kg) in weight over a period of ten years. During the six years previous to admission she had suffered from three spontaneous fractures. Physical examination revealed kyphosis, lordosis and scoliosis, shortening and inversion of the right leg, compression of vertebrae, typical roentgenologic evidence of generalized osteitis fibrosa cystica, adenomatous goiter, generalized arteriosclerosis, hypertension, etc. The serum calcium was 11.3 mg and the phosphorus 3 mg.

At operation two adenomatous nodules were removed but were proved by frozen section to be thyroid tissue. Further search revealed a firm hard nodule in the substance of the right upper pole of the thyroid, which on section proved to be parathyroid tissue. Following parathyroidectomy the serum calcium fell to 10 mg and the phosphorus to 1.9 mg. The patient had a normal convalescence and was discharged sixteen days after operation.

Gross Description—The specimen consisted of a small rounded piece of firm reddish-brown mottled tissue which measured 0.7 cm in diameter. Section revealed a homogeneous tissue with a moist, smooth, reddish-brown mottled surface.

Microscopic Description—The tissue consisted chiefly of dark principal and transitional oxyphil cell forms with but few light principal and mature oxyphil

cells present. The cells showed a slight but definite increase in size, and scattered throughout the tissue were small irregular clumps of giant transitional oxyphil forms which measured up to 30 microns in diameter. These cells contained from 1 to 4 nuclei with prominent nucleoli and measured from 6 to 20 microns in diameter. The intercellular fibrous connective tissue stroma was markedly variable in amount and distribution, being in broad collagenous sheets in some areas and thin stringy wisps in others. The cells were divided into irregular clumps and groups with no appreciable attempt at follicle formation. The vascular supply through the dilated sinusoidal capillaries was irregularly distributed.

Summary Though the majority of the cells were of the transitional oxyphil type, the presence of large numbers of dark principal cells, which were also transitional forms, indicated that this tumor more closely approached the normal gland cell type. Cell hypertrophy with nuclear gigantism and multiplicity was a prominent feature. The fibrous tissue stroma and vascular supply were both increased and irregularly distributed throughout. There was no mitotic activity, but amitotic division was present.

Diagnosis Transitional oxyphil cell adenoma

CASE 4—A white woman, aged 52, was admitted to the New England Deaconess Hospital with a history of intermittent pain in the left epigastric region for sixteen years. Examination revealed an irritable colon and stricture of the anal sphincter. Routine investigation showed an anterolateral bowing of the left tibia, narrowing and rarefaction of the vertebrae, shortening of the right leg and mottling of the bones of the vault of the skull, ilium and sacrum. The serum calcium was 14.1 mg and the phosphorus 2.1 mg.

An exploratory operation was performed, and a tumor the size of a lima bean was found in the position of the right inferior parathyroid. During convalescence the serum calcium fell to as low as 7.8 mg, and a slight degree of tetany was elicited. Recovery was uneventful, and the patient was discharged with normal amounts of serum calcium and phosphorus.

Gross Description—The specimen consisted of an encapsulated bean-shaped piece of soft, yellowish amber-colored tissue which measured 1.5 cm in its greatest diameter. On section the tumor presented a moist homogeneous yellow surface.

Microscopic Description—The majority of the cells in this tumor most closely resembled the pale principal type. Though a few dark principal cells were present, there were no mature oxyphils and only a few transitional oxyphil forms. Many of the cells were of normal size, but the majority had a slightly larger diameter. The cytoplasmic detail was poor, and the cell outline was often indistinct. The cell diameter measured up to 14 microns, and the prominent nuclei measured up to 9 microns. Most of the nuclei were larger than normal, pale staining and finely granular. Only occasionally did a cell contain more than a single nucleus.

There was a slight, irregularly distributed increase in fibrous connective tissue stroma, and though most of the cells presented a protean arrangement, many large alveoli were present. The vascular supply consisted of large sinusoidal capillaries with a marked variability in caliber and distribution. These thin-walled structures were very fragile, and in many areas they had been ruptured by the operative manipulations, producing small areas of fresh hemorrhage throughout the tissue.

Summary The tumor consisted almost entirely of large pale principal cell forms among which no true giant cells were present, and only an occasional example of nuclear multiplicity was noted. There was, however, a fairly well marked

variation in cell and nuclear size. There was a slight irregular increase in fibrous tissue as well as a marked increase in vascularity. No mitotic figures were present, and only an occasional evidence of amitotic division was noted.

Diagnosis Principal cell adenoma

CASE 5—A white woman, aged 45, entered the New England Deaconess Hospital with the complaint of progressive loss of stature and pain in the back and knees increasing in severity for two years. Multiple pains and aches had also been present for six months. Examination revealed marked kyphosis at the level of the eighth dorsal vertebra, knee joints not remarkable and general physical condition good. Roentgenologic evidence of generalized osteitis fibrosa cystica was demonstrated. The serum calcium was 14.4 mg and the phosphorus 2.4 mg.

No tumor was palpable, but when an exploratory operation was performed a large tumor occupying the site of the right lower parathyroid was removed. Convalescence was uneventful with no severe symptoms of hypoparathyroidism. The serum calcium fell to 7.5 mg and the phosphorus to 1 mg six days after operation. When the patient was discharged on the twelfth day the serum calcium had risen to 8.9 mg and the phosphorus to 1.5 mg.

Gross Description—The specimen consisted of an encapsulated tumor mass measuring 3.5 by 2.4 by 1 cm. It was soft and yellowish brown, and showed a small subcapsular hemorrhage. The cut surface was light brown and moist.

Microscopic Description—The tissue was almost entirely composed of transitional oxyphil forms which showed a slight variability in size, with a few large and irregular cells present. The average diameter was about 12 microns with the nuclei averaging about 7.5 microns. The cytoplasm contained a variable amount of granular material ranging from an occasional red granule to a solidly packed granular mass. Nuclear gigantism and multiplicity were present but rare.

There was only a slight irregular increase in the fibrous tissue stroma which divided the cells into irregular groups. A few alveolar structures lined by tall columnar transitional oxyphil forms were noted. These acini contained no secretory product, but some were filled by recent hemorrhage, probably of traumatic origin. The sinusoidal capillaries showed a marked variability of caliber, engorgement and distribution.

Summary The tumor consisted chiefly of transitional oxyphil cells which showed a considerable variability in size. There was a generalized increase in diameter of virtually all cells and nuclei. Nuclear gigantism and multiplicity were present rarely. There was no mitotic activity, but amitotic division was demonstrable.

Diagnosis Transitional oxyphil cell adenoma

CASE 6—A white woman, aged 55, first noted a small symptomless lump in the right breast about one year before death. When first seen at the Huntington Memorial Hospital she had an extensive carcinoma of the breast with metastases to the axillary lymph nodes, the bones of the skull, spine and pelvis and both lungs. She was given a course of roentgen treatments with some relief from pain. Her admission to the Pondville State Hospital was essentially for terminal care. At this institution she was given further radiation treatment but her course was progressively downhill. Roentgen films showed a widespread irregular rarefaction of bone which in view of the primary tumor was interpreted as metastatic tumor lesions. No studies of the blood were done.

Autopsy, performed by Dr Cecil Krakower, revealed the fact that, though several foci of bone metastases were present, most of the osseous lesions were tumor-free. They were diagnosed as generalized osteitis fibrosa cystica. A care-

ful search of the thyroid region revealed a tumor in the position of the right superior parathyroid. The other parathyroids were not noted.

Gross Description—The tumor consisted of a mass of soft reddish-brown mottled tissue which measured 1.7 cm in length and 0.9 cm in its greatest diameter. At one end of the tissue was a small mass about 0.7 cm in diameter.



Fig 2 (case 6)—Parathyroid adenoma, $\times 15$. A photomicrograph of a section of the whole tumor showing two large portions and a rim of normal tissue at the top. Note the size, number and irregular distribution of the vessels.

which was of a more yellowish-brown color and resembled normal parathyroid tissue.

Microscopic Description—The tumor could be conveniently divided into three portions (fig 2). The largest part consisted almost entirely of mature oxyphil cells, the exception being an occasional transitional oxyphil in which there was a

small perinuclear clear space with a slight diminution in cytoplasmic granularity. The oxyphil cells were of a fairly uniform size throughout and measured from 12 to 16 microns in their greatest diameters. The cytoplasm was filled with strongly acidophilic granules which were more closely packed in some than in others. The nuclei varied from 4 to 8 microns in diameter, with the average being about 6 microns. No true giant nuclei were present, but several cells contained 2 or 3 nuclear structures.

There was a slight increase in fibrous tissue stroma, also a slight variation in capillary caliber and distribution. The cells were arranged in solid protean masses, cords and alveolar structures, with many of the latter being large, dilated and cystic in appearance.

The second portion consisted of a smaller mass of cells situated at one end of the section and separated from it by a small amount of fibrous tissue.

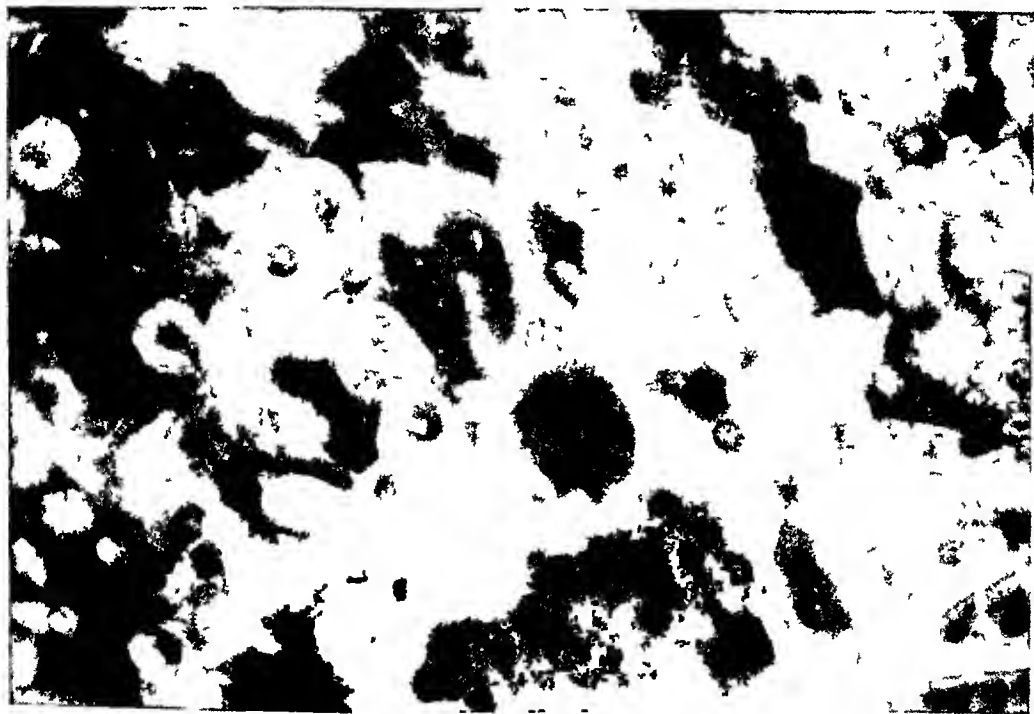


Fig 3 (case 6)—Parathyroid adenoma, $\times 1,500$. The smaller mass is shown. Note the mature and transitional oxyphil cells.

The cells of this area consisted of principal cell forms, throughout which were scattered large and small groups of oxyphils morphologically similar to those in the larger mass (fig 3). The cellular arrangement, fibrous tissue and vascular supply were similar to those already described.

The third portion consisted of a narrow rim of tissue composed of principal cells and separated from the tumor by a small amount of fibrous tissue.

Summary. The main tumor mass consisted of cells which were definitely oxyphilic in type. There was a slight increase in fibrous tissue stroma and vascular supply with irregularity of distribution. Nuclear and cellular gigantism was not present, but multiple nucleated cells were noted. The smaller portion of tissue consisted chiefly of normal pale principal cells with many oxyphils and transitional forms scattered throughout. The outer rim of normal-appearing tissue suggested that this portion represented the original glandular tissue, with the other portions constituting an adenomatous neoplasia. The absence of cellular

compression was, however, not in favor of this assumption. The weight of evidence seemed to favor the diagnosis of adenoma.

Diagnosis Oxyphil cell adenoma

SUMMARY OF REPORT OF CASES

The more interesting features of the cases (table) are as follows:

All the patients were women from 45 to 62 years of age, the average being 52.3 years. Each one presented roentgenologic evidence of fibrocystic bone disease while none had nephrolithiasis. The preoperative serum calcium determinations ranged from 11.3 to 14.4 mg, and it was noted that in the cases with the higher preoperative values the serum calcium fell to lower levels after parathyroidectomy. Though probably of no significance, it is interesting to observe that 5 of the tumors occurred on the right side and 4 in the lower glands. Four of the tumors were classified as transitional oxyphil cell adenomas, 1 as a principal cell adenoma and 1 as a mature oxyphil adenoma.

Considering cases 1, 2, 3 and 5, in all of which the diagnosis was transitional oxyphil cell adenoma, there appears to be a direct relationship between the size of the tumor and the preoperative level of the serum calcium. There is, however, not sufficient evidence to merit the drawing of any conclusions from the observation.

COMMENT

Castleman and Mallory¹ divided benign parathyroid tumors into two groups: adenoma and hyperplasia. By the classification of these workers the cases reported in the foregoing section, with the possible exception of case 6, were all instances of adenoma.

The most prominent feature of all these tumors was the generalized cellular enlargement. Though cells of normal dimensions were seen, the size of the average tumor cell was many times that of the normal. Since the only true estimation of cell size is determined by computation of its volume, a considerable time was devoted to this study. The equation $V = 1/6 \pi D^3$ was used, the value D being estimated as accurately as possible after the study of serial sections of the tumors. In case 1 many of the larger cells were found to be more than eighty times the volume of the normal cell. It was also found that if the tumor were due to a straight hypertrophy of preexisting cells its size could in many cases be accounted for by this hypertrophy.

Hypertrophy alone, however, is not sufficient to explain the many histopathologic aspects. Nuclear multiplicity and gigantism were noted in varying degrees in all the tumors, the number ranging from 2 to 6 and the size up to 20 microns. The nuclear abnormalities are interpreted as indicative of amitotic division, the etiologic factor of which is unknown. Many of the nuclei contained from 2 to 5 prominent large round nucleoli, irregularly distributed (fig. 1B).

Summary of Data on Six Cases of Parathyroid Adenoma

Case	Age, Yrs	Sex	Dura tion of Sypm toms, Yrs	Roentgen Observations	Serum Calcium and Phosphorus				Site	Size, Cm	Tumor	Cells		Nuclei		Cell Volume (n = 267.99 Cu Microns)
					Preoperative		Postoperative					Variation, Microns	Mean, Mi crons	Varia tion, Microns	Mean, Mi crons	
					Cal cium, Mg	Phos phorus, Mg	Cal cium, Mg	Phos phorus, Mg								
1	62	♀	5	+ Bone Changes Renal Stone 0	14.0	2.0	7.8	2.4	LL	3 by 2 by 1.5	A Transitional oxyphil	9-16	13	7-9	8	1,149.76
2	52	♀	Several	+	12.5	2.5	10.9	2.8	RL	1.0 diameter	Transitional oxyphil	7-30	12	6-14	8.5	894.32
3	48	♀	10	+	11.3	3.0	10.0	1.9	RU	0.7 diameter	Transitional oxyphil	7-30	10	6-20	8	523.33
4	52	♀	4	+	14.1	2.1	7.8	3.7	RL	1.5 diameter	Principal cell	7-14	10	4-9	7	523.33
5	45	♀	4	+	14.4	2.4	8.9	1.5	RL	3.5 by 2.4 by 1.0	Transitional oxyphil	8-16	12	6-10	7.5	894.32
6	55	♀	2	+	0				RU	1.7 by 0.9 by 0.9	Oxyphil	12-16	14	4-8	6	1,436.02

In some of the tumors the cells were fairly uniform while in others there was a marked variation within the area of a low power microscopic field. This distribution of cells into protean masses, large and small clumps, cordlike structures and alveoli depended to a great extent on the fibrous connective tissue stroma. All tumors examined showed to some extent an irregular increase in fibrous tissue, this was seen as thickened collagenous sheets in some areas and as thin stringy wisps in others. Although all the aforementioned types of cell arrangement are found in normal glands, the change in stroma is a specific attribute of tumors of the parathyroid. The blood vessels of the normal gland, as previously described, are abundant and of a fairly even caliber and distribution while those of the tumor group showed a marked irregularity in caliber and distribution with large, dilated, engorged sinusoidal capillaries being frequently seen.

Castleman and Malloy¹ stressed the significance of the localized character of the proliferative process as being indicative of neoplasia. These authors stated: "The crux of the argument rests, in our opinion, in the localized character of the proliferative process. Our own experience indicates this is frequently limited not merely to one gland but to a portion of a single gland." They further recorded that they had demonstrated a rim of normal parathyroid tissue on one margin of the tumor in 8 of their 19 cases of adenomas and gave seemingly ample reasons for the paucity of such observations by other workers. Though an effort was made to demonstrate normal parathyroid tissue about the tumors described here we were, with the possible exception of case 6, unable to do so.

As noted, the tumor in case 6 differed from the other tumors in several respects, and its diagnosis as an adenoma is only tentative. The histologic feature most indicative of a neoplastic formation was the narrow rim of normal principal cells, but these cells showed no evidence of compression, and it could not be proved that they did not represent a group of cells displaced from the smaller mass of tissue. There is also the possibility that the latter region represented the original gland and the larger one an adenoma, but the presence of morphologically identical abnormal cells in both portions does not substantiate this assumption. Further, the cordlike arrangement of cells, their relative uniformity of size, shape and structure, the comparative lack of cell hypertrophy and nuclear multiplicity and the more nearly normal distribution of fibrous tissue stroma and vascular supply are all somewhat indicative of hyperplasia. The hyperplasias reported by Castleman and Mallory¹ were generalized, involved all the glands and consisted entirely of principal ballooned or *wasserhelle* type cells. The most significant fact against the diagnosis of the condition in case 6 as a hyperplastic gland is that the other glands were not notably abnormal. It is possible, however, that comparison with the thyroid gland may be helpful, for

it is not uncommon to find a lobe or portion of a lobe to be hyperplastic while the remainder of the gland is relatively normal. Though the parathyroid tissue is commonly divided into four glands, it is assumed that the tissue functions as a unit and hence by analogy with the thyroid a focal hyperplasia of the parathyroid tissue may occur, giving rise to an apparent tumor.

Much remains to be learned about the physiology of the parathyroid gland and the significance of its various component cells. Among the unknown details is the factor or factors which bring about the formation of oxyphil cells. As noted, case 6 was one of carcinoma of the breast with multiple bone metastases as well as generalized osteitis fibrosa cystica. In another case of carcinoma of the breast with bone metastases but no roentgenologically identifiable fibrocystic bone changes, most of one of the parathyroids was occupied by a large nest of oxyphils. These cells were in every respect morphologically similar to those in case 6 and contained the only mitotic figure seen in this study. This mass of oxyphils may be interpreted as representing an early stage of a parathyroid tumor or as an oxyphil cell response to bone destruction. In the latter regard it is of interest to note, and speculate on the possible significance of, 2 cases of carcinoma of the breast with secondary osseous lesions in which the parathyroids showed definitely abnormal masses of oxyphil cells. The functional activity of the various cell types has been previously discussed,⁴ and it is beyond question that the principal cell forms are the primary and essential endocrinologically active cell types. The discovery of a parathyroid tumor associated with a generalized osteitis fibrosa cystica and consisting almost entirely of oxyphils is definite evidence of the functional activity of this cell. The presence of a mitotic figure within an oxyphil is considered proof that these cells are capable of growth activity and are not merely inert structures. The histologic studies of fat, glycogen and mitochondria are recorded elsewhere⁴ and repetition is unnecessary. It is felt that, though admittedly varying in degree, all cells, including the mature oxyphil forms, are capable of elaborating parathyroid hormone.

CRITERIA FOR DIAGNOSIS OF ADENOMA

The detailed study of the tumors described and a review of the literature reveal many interesting points in the diagnosis of parathyroid adenomas, i e., a benign neoplastic growth of the parathyroid tissue.

No constant cell type is found in these adenomas, and though each tumor consists essentially of one cell form, i e., principal cells, transitional oxyphil forms or mature oxyphil cells, it is extremely uncommon to find a parathyroid tumor entirely composed of one cell type. Though parathyroid adenomas are classified into several varieties, e g., transitional oxyphil, there is a tremendous variation in cell size, structure and phase of development from tumor to tumor, area to area, and indeed

within the confines of a single microscopic field. The recognition and correct interpretation of these cell forms require a knowledge of the normal cell and its transitional phases. Cellular and nuclear gigantism are not necessarily present, but these large forms are frequently found. Many cells measure 50 microns in diameter while nuclei 20 microns in diameter are not uncommon. Each cell of an adenoma is, however, usually larger than its normal counterpart, and the average cell is always of greater magnitude. In judging the cell size it is well to remember that the only true criterion is volume.

Multiplicity of nuclei was present in all the cases recorded in this paper. Though this feature is not commonly noted it is felt that a careful search will often, if not always, reveal cells containing from 2 to 6 nuclei. The multiple nuclei are interpreted as indicating amitotic division. Many of the nuclei of the adenoma cells contain from 2 to 5 or 6 large, strongly acidophilic nucleoli.

There is no definite cell arrangement in the parathyroid adenomas. The distribution of cells is somewhat dependent on the location of the fibrous tissue stroma. Thus adenomas are found in which the cells are arranged in protean masses, large and small clumps, cords and acinar structures.

The parathyroid adenomas show a well defined irregularity in amount and distribution of fibrous tissue stroma. In some areas this tissue is abundant and collagenous and in others thin, stringy and barely demonstrable. This stromal change varies in degree in different tumors as well as in different areas within any one adenoma. Similarly there is a well marked variability in the caliber, number and distribution of the sinusoidal capillaries, and these thin-walled delicate structures possess a marked tendency to hemorrhage.

Most adenomas contain very few, if any, fat cells. From the examination of the tumors it is evident that the fat, which is normally present in large quantities in the elderly adult gland, is the first tissue replaced by the tumor growth.

SUMMARY

Six cases of parathyroid tumor are reported. Four of the tumors are diagnosed as transitional oxyphil cell adenoma, one as principal cell adenoma and one as probably oxyphil cell adenoma.

All tumors produced roentgenologically recognizable bone destruction (osteitis fibrosa cystica), but no nephrolithiasis was demonstrable.

Evidence is presented to show that all forms of the parathyroid cell are capable of producing parathyroid hormone.

Several criteria for the diagnosis of parathyroid adenoma are discussed. These are increased size of the individual cells, multiple nuclei, irregularity of stroma and capillary form, and variability of cellular arrangement.

CYANOTIC ATROPHY OF THE LIVER

A WAX MODEL RECONSTRUCTION

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AND

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The changes produced in the liver because of impaired circulation of the blood are well known. Lambert and Allison¹ described several types of lesions produced as a result of chronic passive hyperemia of the liver. These represented different stages in the one process. The cause of the disappearance of the liver cells has been a subject of some discussion. Orth² and others attributed the central necrosis of the liver cells to pressure atrophy. Mallory³ ascribed the necrosis to toxins acting in combination with a poor nutrition of cells. MacCallum⁴ suggested that anoxemia is the important factor in central necrosis of cyanotic atrophy of the liver. Gerlach⁵ attributed the final disintegration of cells to anoxemia and then to pressure.

The well known gross and histologic pattern produced by venous stasis is dependent on atrophy and destruction of the liver cells in the center of the lobules, with replacement by red and white blood cells mixed with some fragments of liver tissue. In the beginning of the change there is nothing more than hyperemia of the central veins and the surrounding sinusoids. As the circulatory impairment increases, there is atrophy of the liver cells in the center of the lobules and hemorrhage. These changes extend for varying distances from the central vein to the periphery of the lobule. The liver cells are sharply demarcated from this zone of blood.

When the venous stasis becomes very severe, such alterations are increased so as to involve a large portion of the lobule. The destruction

From the Norman Bridge Pathological Laboratory of Rush Medical College

1 Lambert, R. A., and Allison, B. R. *Bull. Johns Hopkins Hosp.* **27**: 351, 1916.

2 Orth, J. *Compendium der pathologisch-anatomischen Diagnostik*, ed. 6, Berlin, A. Hirschwald, 1900, p. 545.

3 Mallory, F. B. *J. M. Research* **9**: 455, 1911.

4 MacCallum, W. G. *Textbook of Pathology*, Philadelphia, W. B. Saunders Company, 1930, p. 469.

5 Gerlach, W., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol. 5, pt. 1, p. 91.

of liver tissue does not extend concentrically throughout the lobule. The liver parenchyma in the region of the portal structures remains intact. At certain intervals along the periphery the erythrocytes that have replaced the usual liver parenchyma extend beyond the periphery of the lobule to merge with similar regions of an adjacent lobule or lobules. When this change is marked, the portal triad structures are surrounded by a rim of intact liver parenchyma, and this in turn is encircled by a broad zone of red cells. Because of this alteration, the usual lobule pattern seems reversed, in that gray-brown or yellow tissue appears to be in the center and red tissue at the periphery. This was referred to by Saltykow⁶ as the paradoxical appearance of cyanotic atrophy.

Gerlach⁵ discussed other phases of the changes in chronic cyanotic atrophy of the liver. He concluded that the irregular distribution of the destroyed liver tissue is due to the pressure exerted on the liver cells from the dilated capillaries, in combination with a pressure similarly exerted by the capsule. He also stated that the liver tissue regenerates when chronic cyanotic atrophy is marked, but not to the extreme degree seen in cirrhosis. As far as we know, there have been no studies of the regions of cyanotic atrophy of the liver in all three of its dimensions.

WAX MODEL RECONSTRUCTION

With this in mind, a wax model reconstruction of regions of destroyed liver cells was made.

The liver used was from the body of a man who died in the Presbyterian Hospital, in the service of Dr. Ernest E. Irons, as a result of coronary thrombosis. The important changes found in his body were generalized arteriosclerosis, recent and old thrombosis of the right coronary artery, infarction scars of the posterior wall of the left ventricle and posterior half of the ventricular septum and more recent infarct of the ventricular septum. The liver grossly had the usual appearance of rather marked cyanotic atrophy. Histologically, the parenchyma about the central veins was destroyed for varying distances toward the edge of the lobule and was replaced by erythrocytes and occasional leukocytes, while at the periphery were liver cells in varying degrees of compression and necrosis. A piece of liver tissue 11 by 3 by 18 mm. was cut serially into one hundred and forty-eight sections. Tracings representing a magnification of 30 were made of every third section. A wax model proportional to the sections was made.

In the stained sections the lobules of the liver were cut in the transverse, longitudinal and oblique planes. The regions of atrophy corresponding to the centers of lobules varied in size. In many places they extended beyond the periphery of the lobule and coalesced with similar regions of adjacent lobules. When this fusion was extensive, small portal triad structures were completely surrounded by intact liver tissue, and this in turn was encircled by a zone of atrophy. Commonly, however, in this liver small portal triads were only partially surrounded by such regions.

⁶ Saltykow, S. *Verhandl. d. deutsch. path. Gesellsch.* 5: 104, 1902.

The regions of atrophy represented in figure 1 are fair samples of the changes produced in the liver by the venous stasis. The structure labeled 1 represents the portion of a lobule where liver tissue is absent as a result of chronic cyanotic atrophy. Such changes seem to be greatest at the end where the central vein begins, and from here they proceed through the length of the lobule, decreasing slightly in extent. About the periphery are areas labeled 2, 3, 4 and 5, that represent portions of lobules altered in a manner similar to the changes in 1. Along contiguous sides the regions of cyanotic atrophy of two lobules labeled

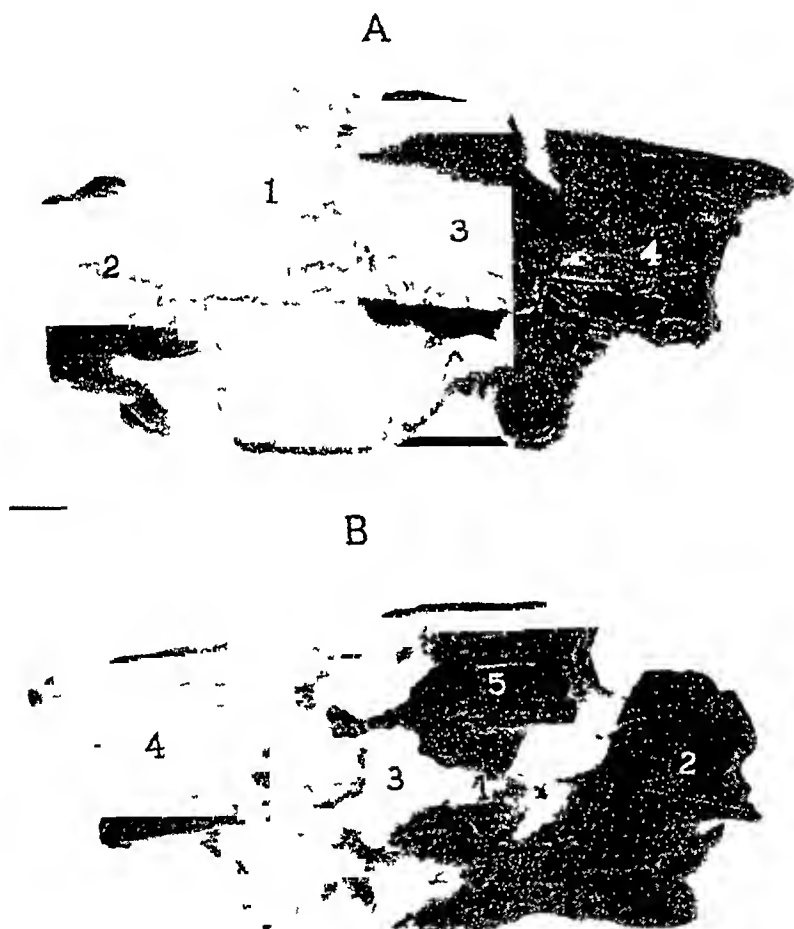


Fig. 1—Wax model showing regions of destroyed liver cells. In *A* the region labeled 1 represents the place in a single lobule where, from end to end, the liver tissue has disappeared. The base of the lobule is at the top. The numerals 2, 3 and 4 indicate places in adjacent lobules from which the liver tissue has completely disappeared. The hole below 3 and other similar regions in *B* are places where liver tissue and portal vessels were still present when the tracings were made. *B* is the other side of the areas seen in *A*. The numeral 3 in this region represents the periphery of a lobule where liver tissue remained. The other dark spots also are places where liver tissue was present in the sections. The numeral 5 indicates the region from which liver tissue had disappeared altogether in a lobule not shown in *A*.

1 and 2 are fused for almost their entire length. These two lobules happen to have parallel long axes. Along another side region 1 has a smooth periphery, without being joined to other similar places of atrophy. In this instance fairly large portal structures are adjacent and prevent coalescence with nearby lobules. Region 3 represents a portion of a third lobule from which the liver tissue has disappeared, and this lobule is directed slightly obliquely to the lobule numbered 1. Fusion occurred only where the two came close enough to be in contact.

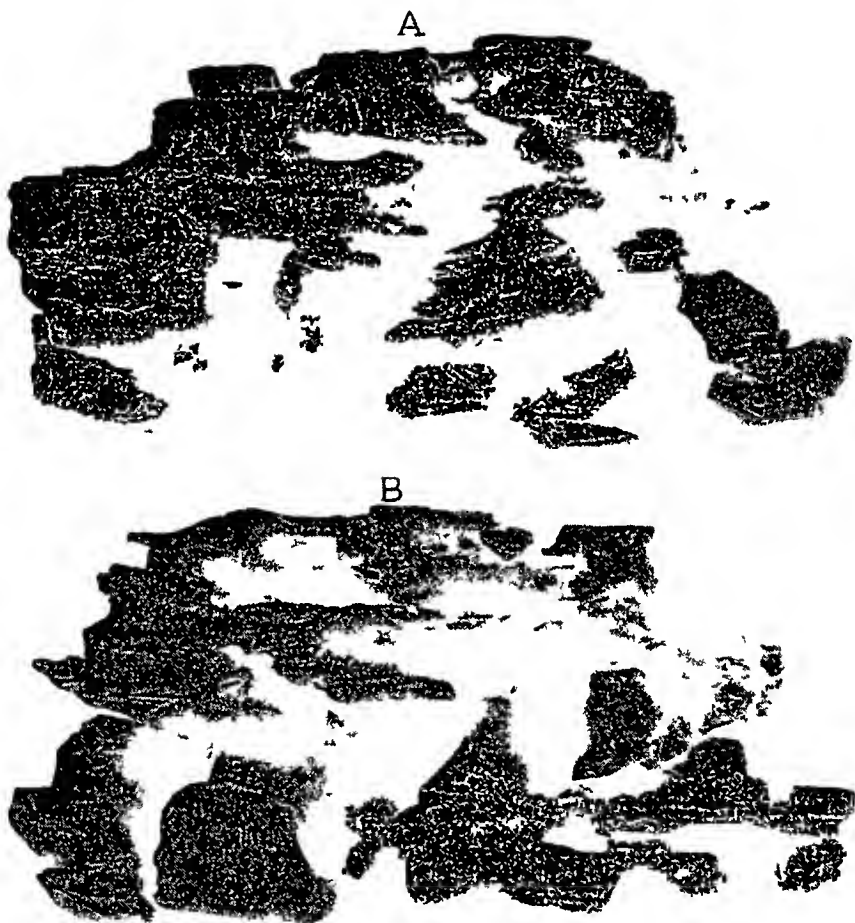


Fig 2—Wax model. In *A* the lighter places are regions of cyanotic atrophy from which the liver tissue has practically disappeared. The darker regions represent the peripheries of lobules where the liver cells, portal veins and accompanying vessels are still present. Altogether there are about thirty lobules. *B* is a photograph of the opposite side of the places illustrated in *A*.

Region 4 is directed obliquely to a considerable degree. On account of its position it is coalesced with region 1 only slightly and with region 3 considerably more. Along another portion of the periphery of the atrophic region 1 coalescence also occurred with one more district of atrophy. This was a lobule which is labeled 5. It is only partly

shown by the model and is also obliquely directed to region 1. In all, about 30 per cent of the periphery of the area of cyanotic atrophy in lobule 1 is continuous with areas of similar atrophy in altogether four different lobules.

The reader should keep in mind that the actual amount of liver tissue destroyed is represented by the model (fig 2) and is far more extensive than would be estimated from the examination of single microscopic preparations. The spaces in the model represent areas occupied by intact liver tissue, including portal triad structures. It is apparent that there are many regions where the districts of destroyed liver tissue have coalesced. Some are broad, others, narrow. The extent of the fusion, as has been pointed out, depends on whether the long axes of adjacent lobules are parallel or at an angle to one another. Those that lie parallel are joined for a considerable distance, others that lie obliquely are united for only a short distance. In some regions of the reconstruction there is a more marked destruction of liver tissues than elsewhere. This is in accord with the ideas expressed on the subject by various writers.

In general, a lobule exhibiting changes of chronic cyanotic atrophy may merge at the periphery with four or five adjacent lobules. The degree of the blending is variable, depending on the position of the adjacent lobules. Those that lie parallel are united without interruption for considerable distances by medium-sized bands of fusion in which the process of destruction is continuous from the center of one to the center of the other lobule. Less than 50 per cent of the periphery of a lobule is involved in the regions of fusion. The damming back of venous blood does not extend beyond the periphery of the lobule, where the portal vein and accompanying vessels are located. Portal structures are barriers and prevent regions of cyanotic atrophy from extending through them. The amount of liver tissue destroyed seems to be greatest in the region where the central vein leaves the lobule.

CENTRAL CIRRHOSIS

For a time, some pathologists⁷ thought that the changes produced in cyanotic atrophy of the liver led eventually to cardiac or central cirrhosis. In some quarters, the idea still prevails. Letulle⁸ discussed the subject and still advocated that the changes produced by chronic passive hyperemia lead to this type of cirrhosis. On the other hand, Gerlach⁵ and others denied that there is such a thing as a cardiac or central cirrhosis.

⁷ Parmentier, E. *Études cliniques et anatomo-pathologiques sur le foie cardiaque*, Paris, G. Steinheil, 1890.

⁸ Letulle, M. *Anatomie pathologique*, Paris, Masson & Cie, 1931, vol. 3, p. 1633.

In the late stages of a long continued and marked passive hyperemia the liver may be firmer than usual and shrunken. The capsule and the walls of the hepatic veins have an increased amount of fibrous tissue. The fibrous tissue in the region of the portal triad structures may seem to be increased but this is relative and not actual. Such fibrous tissue is conspicuous because of the marked destruction of liver tissue.⁹ In places the fibrous trabeculae projecting into the parenchyma from Glisson's capsule may surround groups of lobules, but these changes are not those of true cirrhosis. The color of livers changed by chronic passive hyperemia is different from the color of cirrhotic livers, and also the granular appearance is more pronounced in the latter. Herxheimer¹⁰ stated that with stains in which silver is used to make the stroma conspicuous he failed to find changes in the liver similar to those seen in cases of true cirrhosis.

Piery¹¹ explained the fibrous tissue proliferation in livers having the changes of impaired circulation on the basis of an inflammatory process. In this regard, Dr. E. R. LeCount called our attention to a liver which actually had central cirrhosis. It came from the body of a patient who had chronic hyperplastic tuberculous pleuritis, chronic deforming mediastinitis and obliteration and calcification of the pericardium. This case formed the basis of a report by Dr. J. B. Herrick entitled "Pericarditic Pseudo-Cirrhosis of the Liver."¹²

Camera lucida tracings of stained serial sections were made by Dr. Leila Jackson. The tissue was made up of irregular masses of liver cells, limited by conspicuous fibrous tissue that did not conform to the usual lobule pattern. This fibrous tissue was not prominent about the portal vein. In the stroma that subdivided the lobules of the liver the blood vessels were not associated with bile ducts. These vessels were followed from section to section, and their continuity with larger hepatic veins was demonstrated. The liver cells adjacent to the dense fibrous tissue were atrophic, and the sinusoids were distended with erythrocytes. The liver tissue adjacent to the portal triad was healthy. Sketches of some of the tracings made from serial sections are shown in figure 3. Obviously, the lobules of the liver were subdivided by fibrous tissue, and included in this stroma are veins which, when traced, were seen to empty into larger ones. The increase of this fibrous tissue was mostly in the center and not in the periphery of the lobules. Such a change may be called central cirrhosis.

9 Karsner, H. T. Human Pathology, ed. 2, Philadelphia, J. B. Lippincott Company, 1929, p. 683.

10 Herxheimer, G. Beitr. z. path. Anat. u. z. allg. Path. **43**: 284, 1908.

11 Piery, H. Lyon med. **50**: 997, 1909.

12 Herrick, J. B. Tr. Chicago Path. Soc. **5**: 71, 1902.

The condition, however, may not be a result of chronic passive hyperemia of the liver. The disease was explained by a retrograde lymphangitis from the tuberculosis of the mediastinum and pleura following along the inferior vena cava and hepatic veins, but without obvious tuberculous granulation tissue, because the tuberculosis was hyperplastic and almost totally devoid of foci of caseation. Such chronic inflammation is common in the peripheries of areas where pro-

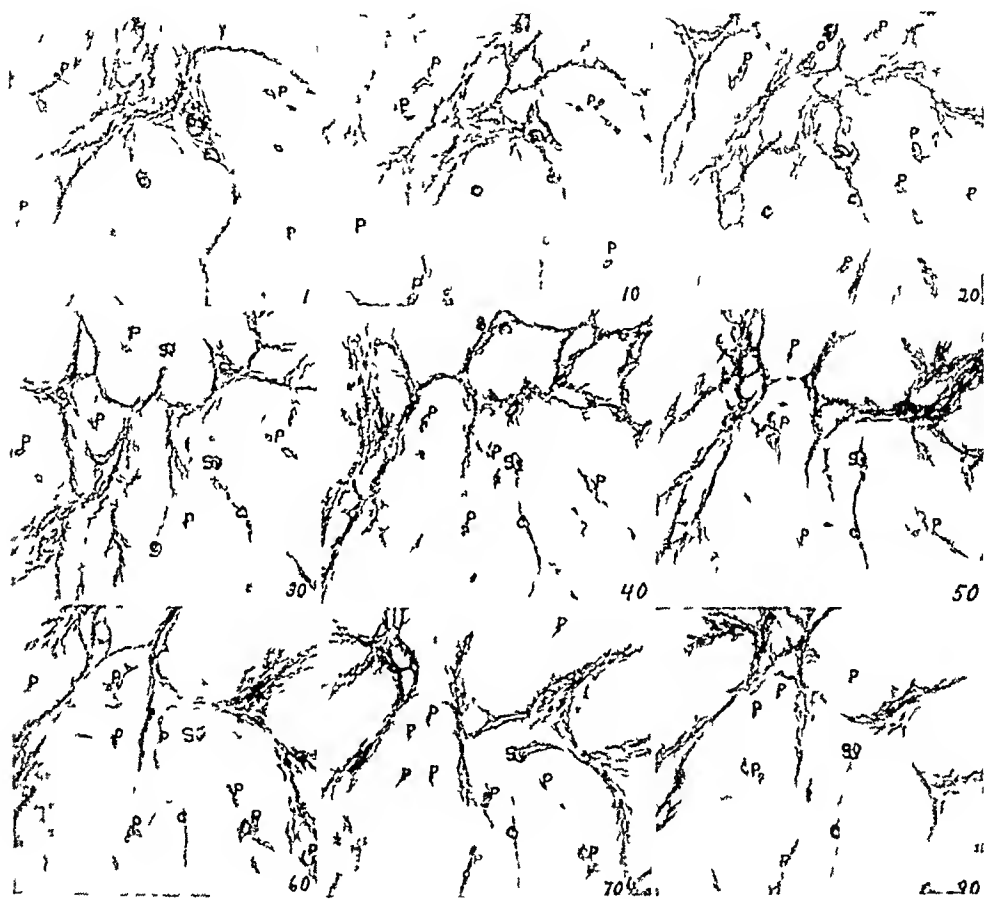


Fig 3—Tracings made from serial sections of the liver, indicating the distribution of the fibrous tissue within lobules. *P* denotes the position of the portal structures, *C*, the central veins, and *S*, the sublobular veins

nounced disease has existed for some time. It is well known that lymphatics from the liver accompany the hepatic veins and ascend into the thorax along the inferior vena cava.¹³

SUMMARY

A wax model reconstruction of the regions of cyanotic atrophy of a liver was made. This method reveals that the amount of liver tissue

¹³ Delamare, G. The Lymphatics, Chicago, W. T. Keener & Co., 1904, p. 201

destroyed is more than it appears to be in stained sections. The regions of atrophy of an individual lobule extend beyond the periphery to coalesce with similar tissues in four or five other lobules, involving less than 50 per cent of the peripheries of such units. Whether the fusion between the regions of cyanotic atrophy of two adjacent lobules is extensive or not depends on the position of these lobules. The fused regions of cyanotic atrophy of adjacent lobules are largest when the latter have parallel axes.

An example of central cirrhosis of the liver is given, and this disease is explained by a retrograde lymphangitis from tuberculosis of the mediastinum and pleura extending along the inferior vena cava.

VITAMIN A DEFICIENCY

IN SPITE OF ADEQUATE DIET IN CONGENITAL ATRESIA OF BILE DUCTS AND JAUNDICE

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The association of severe protracted jaundice with the various manifestations of what is now recognized as vitamin A deficiency was well known to the continental clinicians of the last century. Their reports,¹ while they apparently demonstrated some connection between icterus and hemeralopia, xerosis and keratomalacia, cannot be analyzed critically now because of the omission of any reference to the diets the patients were receiving. It is well known that patients with hepatic disease usually experience anorexia and frequently nausea, vomiting or diarrhea as well. The occurrence of vitamin A deficiency in the cases reported in the earlier literature may have been due to malnutrition associated with these gastro-intestinal disturbances.

For a period of about twenty years in the early part of the present century no additional reports of cases appeared. There was, however,

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1 Bamberger, H. Krankheiten des chylopoetischen Systems, in Virchow, R. Handbuch der speziellen Pathologie und Therapie, Stuttgart, Ferdinand Enke, 1855, vol 6, pt 1, p 522. Junge, E. Verhandl d phys-med Gesellsch in Wurzburg 9 219, 1859. Frerichs, F. T. Klinik der Leberkrankheiten, Braunschweig, F Viewig & Sohn, 1858, vol 1, p 116. Murchison, Charles. Clinical Lectures on Diseases of the Liver, Jaundice and Abdominal Dropsy, New York, William Wood & Company, 1868, p 372. Fumagalli, A. Ann di ottal e clin ocul 2 471, 1873. Kohn. Rec d'opht 1 185, 1874. Straus, I. Des ictères chroniques, Paris, J-B Bailliere & fils, 1878, p 104. Gorecki, X. Praticien 3 427, 1880. Mouly, P. Contribution a l'etude de l'hemeralopie dans les affections hepatiques, These de Paris, no 217, 1881. Parinaud. Arch gen de med 7 403, 1881. Raymond. Praticien 4 497, 1881. Cornillon, J. Progres med 9 157, 1881, 10 439, 1882. Weiss. Mitth a d ophth Klin, Tubingen 3 89, 1882. Leber, T. Arch f Ophth 29 224, 1883. Hirschberg, J. Berl klin Wchnschr 22 364, 1885. Vincent, G. L'oeil et le foie, These de Lyon, 1892. Moauro. Ann di ottal e clin ocul 26 554, 1893. Baas, K. L. Arch f Ophth 40 212, 1894, Munchen med Wchnschr 41 629, 1894. Thompson, J. T. Brit M J 2 597, 1894. Hori. Arch f Augenh 31 393, 1895. Levi, L. Presse med 4 165, 1896. Elschmig, A. Wien med Wchnschr 49 842, 1899. Purtscher. Arch f Ophth 1 83, 1900. Volbracht, F. Ztschr f Heilk 24 359, 1903. Spassky. Russk vrach 4 693, 1906. Groenouw, A, in von Graefe, A, and Saemisch, T. Handbuch der gesamten Augenheilkunde, ed 2, Leipzig, Wilhelm Engelmann, 1906, vol 11, pt 1, p 83.

an increase of interest in the subject with the appearance of the publications of Wright² and Wagner³. In these, as in the older reports, there was no definite information as to the adequacy of the dietary regimen of the patients.

That there is a specific connection between hepatic disease with icterus and vitamin A deficiency became apparent when Blegvad⁴ in 1923 and Bloch⁵ in 1924 called attention to the signs and symptoms of this deficiency in three patients who were receiving diets rich in vitamin A. Blegvad's patient experienced relief of the symptoms of deficiency only after the intramuscular administration of a cod liver oil concentrate. The patient described by Bloch who recovered spontaneously did so only after the subsidence of the icterus. Owen and Hennessey⁶ and Blackfan and Wolbach⁷ reported additional instances of vitamin A deficiency with severe jaundice in patients receiving adequate amounts of vitamin A. All these authors considered the vitamin A deficiency to be secondary to defects in the absorption of fat associated with jaundice.

Severe jaundice with complete, or almost complete, exclusion of bile from the small intestine was common to all the cases reported in the literature. Marked enlargement of the spleen and liver was noted in most instances. The patients with this syndrome were described as having Hanot's cirrhosis, hypertrophic cirrhosis of the liver with icterus gravis or biliary cirrhosis. A few cases of a high grade of obstructive jaundice due to malignancy or to cholelithiasis with secondary suppurative or cirrhotic changes in the liver are also found among those reported. In all instances in which it was possible to determine the time relationship between the onset of the jaundice and the appearance of the symptoms of deficiency, it was apparent that jaundice always came first. The interval before the appearance of the symptoms of deficiency varied from weeks to months in children and from months to years in adults.

In recent years Wolbach and Howe⁸ have defined the essential pathologic picture in cases of vitamin A deficiency, other observers⁹

2 Wright, R. E. Statistical and Professional Report of the Government Ophthalmic Hospital, Madras, 1921-1922, *Brit J Ophth* **6** 164, 1922, *Lancet* **1** 800, 1931.

3 Wagner, R. *Wien med Wchnschr* **74** 1108, 1924.

4 Blegvad, O. *Ugesk f læger* **85** 942, 1923.

5 Bloch, C. E. *Am J Dis Child* **28** 659 (Dec) 1924.

6 Owen, H. B., and Hennessey, R. S. F. *Tr Roy Soc Trop Med & Hyg* **25** 367, 1932.

7 Blackfan, K. D., and Wolbach, S. B. *J Pediat* **3** 679, 1933.

8 (a) Wolbach, S. B., and Howe, P. R. *J Exper Med* **42** 753, 1925, (b) **57** 511, 1933, (c) *Arch Path* **5** 239, 1928.

9 Goldblatt, H., and Benischek, M. *J Exper Med* **46** 699, 1927. Tilden, E. B., and Miller, E. G. *J Nutrition* **3** 121, 1930. Wolfe, J. M., and Salter, H. P. *ibid* **4** 185, 1931. Thatcher, H. S., and Sure, B. *Arch Path* **13** 756, 1932.

have corroborated these observations in animals. Wilson and DuBois¹⁰ described a similar lesion in children. The structural effects of vitamin A deficiency are characterized by atrophy of the epithelium of various organs and replacement with a stratified keratinizing epithelium, the metaplastic cells arising in foci deep in the epithelium close to the basement membrane. The formation of the keratinizing epithelium begins simultaneously in many foci. The first change that is demonstrable is the appearance of clusters of cells containing large pale nuclei surrounded by deeply basophilic cytoplasm. These cells increase by mitotic division and soon commence to show keratinizing changes. As they increase in size, the clusters of cells undermine the preexisting epithelium. There is also some extension into the supporting tissues. The keratinizing epithelium which replaces the atrophic normal epithelium in the various organs is identical in all locations. In infants the earliest appearance of keratinizing metaplasia is in the trachea and bronchi. The epithelium of the renal pelvis is usually next involved. The salivary glands, pancreas, uterus, thymus, esophagus, glands of the nasopharynx, bladder, prostate, seminal vesicles and para-ocular glands may also show typical changes. Keratotic changes in the cornea appear late in the disease, far advanced metaplasia may be present in various internal organs with no discernible abnormality in the cornea.

The present article is based on a postmortem study of eleven infants with congenital atresia of the bile ducts. All of them had received diets adequate in vitamin A, and none presented clinical evidence of xerosis or keratomalacia during life.

Microscopic changes diagnostic of vitamin A deficiency, as defined by Wolbach and Howe,⁸ were observed in six of the eleven infants, and the clinical histories and autopsy observations in these six cases are presented. In each of these cases the family history was irrelevant.

REPORT OF CASES

CASE 1—An 8 month old white boy was admitted to the hospital on Aug 13, 1931, because of jaundice. Delivery was normal. Jaundice was noted at birth, with white stools and brown urine, and grew steadily worse. Two weeks before admission anorexia developed, and the infant commenced to lose weight. In a week he became dyspneic. He was breast fed during the first month of life and thereafter was given formulas with added fruit juices, vegetables and cod liver oil for several months.

The infant was fairly well developed, poorly nourished, dyspneic and markedly icteric, with edema of the eyelids. A rachitic rosary was noted. The abdomen was markedly distended, with prominent superficial veins. The liver was felt as a hard nodular mass 4 cm below the costal edge. The spleen was palpable in the left flank as a mass the size of an orange. There was shifting dullness in the flanks.

¹⁰ Wilson, J. R., and DuBois, R. O. *Am J Dis Child* 26:431, 1923.

The icteric index was 325, with a quantitative van den Bergh reaction of 77. A direct van den Bergh reaction was obtained.

An exploratory laparotomy was made on the third day after admission and revealed marked ascites, splenomegaly, hepatomegaly and atresia of the bile ducts. The infant died the next day.

At autopsy, five and one-half hours post mortem, the liver was enlarged, weighing 287 Gm., firm and dark green. The surface was extremely nodular and cirrhotic. The capsule was thickened. Cut surfaces showed strands of connective tissue running throughout the organ, most prominent in the portal areas. There was marked biliary stasis. The gallbladder was represented by a small tab of fibrous tissue lying in the gallbladder fossa. A fine lumen was present in it. No cystic or common bile duct was demonstrable. The spleen was markedly enlarged, weighing 117 Gm., and was fairly firm. Many subcapsular petechial hemorrhages and a slight increase in fibrous tissue were noted.

In addition, intense generalized icterus, slight edema of the lower eyelids, a recent right rectus incision, 75 cc. of bloody fluid in the abdomen, numerous petechial hemorrhages in the pericardium, dependent bronchopneumonia of both lungs and marked rachitic changes in the long bones and ribs were noted.

The observations on which the diagnosis of vitamin A deficiency was made were as follows. There was marked metaplasia of the epithelium of the trachea with complete replacement of the pseudostratified epithelium by stratified squamous cells. Slight keratinization of this epithelium was observed. The epithelium of the renal pelvises showed moderate metaplasia and slight keratinization. There were early metaplastic changes in the pancreatic ducts represented by foci of squamous cells undermining the normal epithelium. The rest of the microscopic examination confirmed the macroscopic observations.

Comment—The child presented microscopic changes in the trachea, renal pelvises and pancreatic ducts indicative of the presence of a moderate degree of vitamin A deficiency in spite of the fact that an adequate diet plus cod liver oil had been received.

CASE 2¹¹—A white girl aged 5 months was admitted on Dec. 31, 1931, because of jaundice. Delivery was normal. Clay-colored stools and dark brown urine were observed three days after birth. Icterus was first noted when the infant was 3 weeks old and increased steadily thereafter. The child was breast fed for two weeks and then was given various formulas containing milk, dextrimaltose with vitamin B and water, all of which were taken well. The infant received 1 teaspoonful of orange juice daily and 5 drops of viosterol twice daily, beginning at the age of 1 month. About a month before admission the infant's left ear commenced to discharge and continued to do so for about a week. Several days before admission she appeared to be feverish and vomited several times.

Examination on admission revealed a deeply icteric, poorly developed and poorly nourished infant. She was entirely normal except that the liver was felt as a hard mass 2½ fingerbreadths below the left costal margin. The stool was clay-colored.

The icteric index was 125 and 180, respectively, on two occasions. The van den Bergh reaction was direct. The quantitative van den Bergh reaction was 50 and 36, respectively, on two occasions.

11 This is case 11 of the series reported by Blackfan and Wolbach.⁷

The patient was given a diet of fat-free milk with 50 per cent Karo. She also received orange juice. One grain (0.065 Gm) of oxgall was given three times a day. The day after admission the temperature rose to 103 F. The next day, after both ear drums were lanced, the temperature returned to normal. The following week a similar series of events occurred. On Jan 12, 1923, an exploratory laparotomy was performed. No gallbladder or extrahepatic ducts were demonstrable. The infant was still receiving fat-free milk and oxgall. On the seventh postoperative day the wound separated and was resutured. Death occurred three days later, the infant being 6 months old.

At autopsy, sixteen hours post mortem, the liver was increased in size, weighing 240 Gm, was much firmer than normal and was dark green. The surface was finely nodular, and the capsule appeared thickened. Cut surfaces showed pronounced biliary stasis and irregular scarring, the latter being especially marked in the portal areas. Deep in the gallbladder fossa a small sac, 1 cm in diameter, which contained dark green inspissated bile, was noted. It continued upward toward the liver as a slightly dilated hepatic duct. No ducts were demonstrable below the sac passing toward the duodenum. The ampulla of Vater, while patent toward the pancreas, had no demonstrable connection with the liver. The spleen was enlarged, weighing 30 Gm, and was abnormally firm. Cut surfaces showed an increase in the amount of fibrous tissue.

The other macroscopic observations included a recent right rectus incision, 30 cc of bloody amber fluid in the abdomen, fibrinous peritonitis, adhesions between the omentum, the body wall and the gallbladder fossa, small adhesions between several loops of bowel and the operative wound, dependent bronchopneumonia of both lungs and marked generalized icterus.

The diagnosis of vitamin A deficiency was based on the fact that the Hassall corpuscles of the thymus were greatly enlarged and were lined by keratinizing squamous cells. The pseudostratified tracheal epithelium showed marked metaplastic changes, having been converted into stratified squamous epithelium. The epithelium of the renal pelvis also presented striking metaplasia. The normal transitional epithelium had been converted into thickened keratinizing stratified squamous epithelium. The underlying connective tissue showed acute and chronic inflammatory cellular infiltration. The epithelium of the esophagus was thickened and hyperkeratotic, and the ducts of its glands showed marked metaplasia of the lining cells.

The other microscopic observations confirmed the macroscopic diagnosis. There were, in addition, acute splenitis, a small blood cyst on the mitral valve, mesenteric lymphadenitis, hemorrhages in the lungs, spleen and parathyroid glands, and evidence of rickets and possibly of early scorbutus.

Comment—This 6 month old infant showed evidence of marked vitamin A deficiency post mortem, but at no time was there any clinical evidence of it. She had received an adequate diet until a short time before death. The fact that she received a diet of fat-free milk for a period of several weeks before death may account in part for the severity of the changes due to the avitaminosis. The possible effect of the oral administration of oxgall in this case cannot be estimated.

CASE 3—A white girl aged 5 weeks was admitted on July 6, 1932, because of jaundice.

There had been difficult instrumental delivery. Jaundice was noted three days after birth and had persisted. The stools were always clay-colored. She was nursed for two weeks and was then given a formula. She took nourishment well and gained weight until a week before admission, when the weight began to decrease.

Examination revealed a fairly well nourished, deeply icteric infant. She appeared to be normal except that the liver was palpable $2\frac{1}{2}$ fingerbreadths below the costal margin.

The stool contained no bile. A direct serum van den Bergh reaction was obtained. The quantitative van den Bergh reaction was 55, and the icteric index, 325.

The infant was given a mixture of whole milk, 50 per cent, Karo and water. She also received 10 drops of haliver oil daily. On July 30, exploratory laparotomy revealed the absence of the extrahepatic biliary passages and atrophy and fibrosis of the gallbladder. The infant recovered quickly and was discharged on August 10. While at home she was given the same diet that she had had in the hospital. She took her feedings well and gained weight for about one month. After that she failed to gain, probably because of a diminution in appetite. The jaundice, with the clay-colored stools and dark urine, continued, and she was readmitted on November 25 because of rapid swelling of the abdomen during the preceding three days.

Examination showed that the abdomen was markedly distended, with dilated superficial veins, shifting dullness and a fluid wave.

A second laparotomy was performed on November 29. No bile duct could be demonstrated. The patient died the next day, at the age of 6 months.

Autopsy, six hours post mortem, showed that the liver was enlarged, weighing 352 Gm. It was firm and greenish gray. On its surface were fine nodules from 2 to 4 mm in diameter. Cut surfaces showed marked dilatation of the biliary passages. The markings were distorted. The gallbladder was represented by a thin plaque of fibrous tissue in the gallbladder fossa. The duodenum was adherent to the fibrous tissue on the under side of the liver. No common bile duct was demonstrable. The transverse colon and several loops of jejunum were bound down to the hilus of the liver with dense fibrous adhesions. The spleen was enlarged, weighing 50 Gm, and had a somewhat irregular surface. Intense generalized icterus, slight edema of the eyelids, a small amount of orange fluid in the peritoneal cavity, a recent right rectus incision and patchy atelectasis in both lungs were also noted.

The features diagnostic of vitamin A deficiency were the early metaplastic changes in the epithelium of the renal pelvis. In some areas there were foci of stratified squamous cells undermining the normal lining cells. The rest of the microscopic examination confirmed the macroscopic observations and revealed, in addition, a moderate amount of diffuse hemorrhage into the alveoli of the lungs with a small amount of inflammatory exudate in some areas, congestion and edema of the lungs, acute splenitis and early rachitic changes in the long bones.

Comment—This 6 month old infant showed slight early changes indicative of vitamin A deficiency in spite of having received an adequate diet plus haliver oil.

CASE 4—A white girl aged 8 weeks was admitted on Oct 25, 1932, because of jaundice. The infant's mother had been well until the middle of the seventh month of pregnancy, when symptoms of toxemia developed. She was taken to

the hospital two weeks later, and labor was induced. The infant was one of twins, weighing only 5 pounds and 1 ounce (2,300 Gm) at birth. She nursed well and received, in addition, complementary feedings of whole milk, Karo and water. She gained as rapidly as did her normal twin. However, jaundice was observed in the infant at birth and persisted until the time of admission. The stools were clay-colored, and the urine was dark brown at all times.

She appeared to be a poorly developed but well nourished, jaundiced infant. The liver was felt 2 fingerbreadths below the costal margin, and the spleen was just barely palpable.

The icteric index was 100.

Exploratory laparotomy was performed on November 18, and a fibrosed atrophic gallbladder was observed. The cystic and common ducts could not be demonstrated. Following the operation the temperature soon fell to normal, remaining at that level for several days, after which it rose again. In addition to her diet the patient received 8 grains (0.52 Gm) of oxgall daily. She nursed well but vomited occasionally. The icteric index persisted at 100. Several days before death cough, dyspnea and signs of pneumonia developed. She died on December 10, at the age of 12 weeks.

At autopsy, seven hours post mortem, the liver was normal in size but fairly firm and presented a finely nodular surface. The right lobe was adherent to the anterior abdominal wall, while the stomach, duodenum, ascending colon and hepatic flexure were adherent to the hilar region. The capsule of the liver was slightly thickened. Cut surfaces showed increased fibrous tissue about the lobules of the liver, the latter appearing diminished in size. The gallbladder was represented by a fibrous, elongated, lumenless structure. No external biliary ducts were demonstrable. The spleen was of normal size. There was marked generalized icterus, and numerous small scattered areas of atelectasis were present in the lungs.

The evidences of vitamin A deficiency revealed by microscopic examination of the tissue were as follows. The renal pelvises showed from slight to moderate metaplastic changes in the epithelium with early keratinization of the lining cells. The pancreatic ducts also showed some undermining of the epithelium by metaplastic cells.

The rest of the microscopic examination confirmed the macroscopic observations and revealed, in addition, bronchitis, bronchopneumonia, hypoplasia of the bone marrow, petechial hemorrhages and hemosiderosis of the spleen and early rickets.

Comment—This 12 week old infant showed slight evidence of vitamin A deficiency post mortem in spite of having received an adequate diet. It is of interest that, in spite of the icterus, her growth and development had paralleled that of her normal twin. Factors that possibly were responsible for the early development of vitamin A deficiency in this case were the toxemia of the mother, the prematurity of the infant and the fact that she was one of twins and therefore puny. The febrile postoperative period, which lasted for several weeks, may also have been responsible in part. It is not possible to estimate the effect of the administration of oxgall in this case.

CASE 5—A Jewish boy aged 4 months was admitted on Jan 17, 1933, because of jaundice. He had otitis media six weeks before admission. Jaundice was

noted three days after birth and became progressively worse. White stools and dark brown urine were noted from the first. The infant was breast fed and received, in addition, complementary feedings of a mixture of milk, water and dextrimaltose. He had vomited a good deal but grew and developed normally. When he was 6 weeks old, enlargement of the liver was noted. When he was 3 months old, this was again observed by a physician, who prescribed a preparation of oxgall and increased the feedings.

Examination revealed a well developed, well nourished, deeply jaundiced infant. He was apparently normal except that the skin bore several furuncles, and the liver was enlarged so as to reach half-way to the iliac crest. The stools were grayish white.

The icteric index varied between 75 and 200. The van den Bergh reaction was diphasic.

The patient was given a transfusion of 125 cc of citrated blood shortly after admission. Following that the icteric index rose from its initial level of 75 to 225. He was given fat-free milk, orange juice and water, all of which he took well. Beginning January 26 he was also given 6 grains (0.4 Gm) of calcium glucoside and 20 drops of haliver oil daily. The temperature was normal until after the transfusion, when it rose to 103 F, falling irregularly to 100 F after four days. At that time he showed signs of bronchitis. The temperature fluctuated between 97 and 99.5 F thereafter.

On February 1 an exploratory laparotomy was performed, and the gallbladder was observed to connect normally with the duodenum. The gallbladder did not contain bile. The jaundice, with dark urine and clay-colored stools, persisted, but the infant took nourishment well and did not vomit. On February 4 the wound ruptured and was resutured, following which a cough, râles in the chest and a temperature of from 102 to 103 F developed. Nevertheless the infant ate well and the cough and fever soon subsided. The wound healed, and he slowly gained weight. On March 21 laparotomy was again performed. No hepatic duct was demonstrated. The gallbladder was removed. There was only a slight post-operative reaction, and the infant's progress was good for a time. He ate well and gained weight slowly. However, on March 7 the jaundice became more severe, the abdomen became distended, and edema of the legs appeared. On March 14 1,300 cc of ascitic fluid was removed. From that time on the infant's condition became worse, and he died on March 23, at the age of 7 months.

At autopsy, ten and one-half hours post mortem, the liver was somewhat enlarged and firm. The surface was finely nodular, the nodules measuring from 1 to 2 mm in diameter. The capsule was thin. The under surface of the liver was adherent to the duodenum and the hepatic flexure, and the liver was a dark mottled green, the mottling being due to an increased amount of perilobular connective tissue. There was marked biliary stasis, the bile ducts appearing prominently. The gallbladder was not demonstrable. The common bile duct was present and admitted a fine probe. No left hepatic duct was demonstrable. The right hepatic duct existed as a fibrous cord, 11 mm long. The common duct was patent into the duodenum but had no connection with the liver. The spleen was larger and firmer than normal, and cut surfaces showed accentuation of the fibrous markings. An accessory spleen, 14 by 12 by 8 mm, was present at the hilus. It, too, showed an increased prominence of fibrous tissue.

Marked generalized icterus, recently healed scars resulting from a right rectus incision, 75 cc of clear amber fluid in the abdomen, dense adhesions involving the upper portion of the ileum, jejunum, hepatic flexure, round ligament of the liver

and omentum, edema and congestion of the mucosa of the gastro-intestinal tract, and congestion of the kidneys were noted. The thoracic organs were not examined.

The diagnosis of vitamin A deficiency was based on the moderate metaplastic changes observed in the epithelium of the renal pelvis. There was complete replacement of the normal epithelium by stratified squamous cells in some areas and only undermining of the normal epithelium by foci of metaplastic cells in others.

The rest of the microscopic examination confirmed the macroscopic observations and revealed, in addition, fatty changes in the renal tubules and acute splenitis.

Comment—This 7 month old infant showed evidence of a moderate degree of vitamin A deficiency in spite of having received an adequate diet plus haliver oil.

CASE 6—A 6 month old white girl was admitted on March 8, 1934, because of jaundice, which was noted at birth and was of unvarying intensity thereafter. The stools were clay-colored and sometimes streaked with blood. The urine was always dark orange. There was frequent vomiting of recently eaten food during the first three months of life but none after that. A slight cough was noted occasionally. The infant was breast fed for one month and was then given a mixture of whole milk, water and Karo. She refused cod liver oil and orange juice. There was little gain in weight after the first few weeks of life.

Examination revealed a fairly well developed, poorly nourished, icteric infant with a large, firm liver and a palpable spleen.

The stool revealed an absence of bile and the presence of occult blood. The icteric index was 60. The van den Bergh reaction was direct, quantitatively it was 2.35 units.

The patient was given a mixture of 2 per cent milk, 50 per cent dextrose and water and also received 2 ounces (60 cc) of orange juice and 10 drops of haliver oil daily. She took these feedings well. On March 17 she was operated on and died on the operating table.

At autopsy, two hours post mortem, the liver appeared smaller than normal and was dark green. The surface was rough and finely nodular. Cut surfaces presented an increased amount of periportal connective tissue. A strand of connective tissue representing an atrophic gallbladder was observed in the fossa of the gallbladder. No common or cystic ducts could be demonstrated. The spleen was enlarged, weighing 75 Gm. Its capsule presented several patches of thickening anteriorly.

Bilateral craniotabes, rachitic changes in the ribs and long bones, a recent right rectus incision, marked generalized icterus, a few cubic centimeters of icteric fluid in each pleural cavity, some hypertrophy of the right ventricle of the heart, a moderate amount of emphysema of the lungs, marked edema of the mucosa of the stomach and a few small acute ulcers in the ileum were noted.

The microscopic observation on which the diagnosis of vitamin A deficiency was made was the presence of a slight or moderate amount of metaplastic keratinizing epithelium undermining the normal lining cells of the renal pelvis.

The rest of the microscopic examination confirmed the macroscopic observations and revealed, in addition, slight early bronchopneumonia.

Comment—This 6 month old infant showed evidence of a slight degree of vitamin A deficiency in spite of having received an adequate diet

OBSERVATIONS

Postmortem studies of eleven infants with congenital atresia of the bile ducts were made. Although these infants had received an adequate diet and, in some instances, vitamin A in some form in addition, microscopic examination demonstrated vitamin A deficiency in six of them. All but one of the infants who showed evidence of the avitaminosis were 6 months of age or older. The one exception was a premature infant whose mother showed symptoms of toxemia before delivery. The infants in whom no histologic evidence of the vitamin deficiency was found were 6 months old or younger (table).

Vitamin A Deficiency in Cases of Congenital Atresia of the Bile Ducts

Severity of Vitamin A Deficiency	Age of Infant	Case
Marked	6 months	2
Moderate	8 months	1
Moderate	7 months	5
Slight	6 months	6
Slight	12 weeks	4
Very slight	6 months	3
None	5 weeks	
None	6 weeks	
None	8 weeks	
None	9 weeks	
None	6 months	

COMMENT

As long ago as 1857, Althof and Muller¹² described the appearance of keratomalacia in two dogs with biliary fistulas which were kept alive for four years. Dolganoff¹³ in 1897 tied off the common ducts in dogs and noted the appearance of the ocular signs of what is now recognized as vitamin A deficiency.

The manner in which obstructive jaundice acts to make vitamin A unavailable to the body is only partially understood. Blegvad's⁴ experience with his patient who responded to the intramuscular administration of a special extract of cod liver oil but not to the oral administration of the oil led him to express the belief that the absence of bile from the small intestine in cases of severe obstructive jaundice in some way prevents the absorption of the fat-soluble vitamin A.

¹² Althof, H., and Muller, H. *Wurzb med Ztschr* 2:349, 1861.

¹³ Dolganoff, W. *Arch f Augenh* 34:196, 1897.

More recently Schmidt and his co-workers¹⁴ have shown that dogs with an induced vitamin A deficiency and exclusion of bile from the small intestine, due either to obstruction of the common duct or to formation of fistulas between the gallbladder and the colon, could not be cured of disease due to vitamin A deficiency with cod liver oil or carotene given orally. The signs of the deficiency in these animals could, however, be made to disappear following the intramuscular administration of vitamin A. Similar results were obtained by these investigators by giving carotene or cod liver oil by mouth together with bile salts.

However, it is not certain that the failure of icteric patients to absorb vitamin A is related directly to the faulty absorption of fat which occurs in jaundice, since neither vitamin A nor its precursor, B-carotene, is a fat, the former is an alcohol¹⁵ and the latter a hydrocarbon¹⁶. Both are readily separated from the fats with which they are found by the process of saponification, carotene and vitamin A remaining in the unsaponified fraction. The part played by bile in the absorption of complex hydrocarbons and their alcohols has not yet been elucidated. It has been shown,¹⁷ however, that both bile and certain pancreatic enzymes are necessary for the absorption of cholesterol, a substance chemically allied to vitamin A and carotene. The absorption of cholesterol and the absorption of fat are, however, entirely independent of each other,¹⁷ it may well be that the same is true of the absorption of fat and of vitamin A.

Some degree of vitamin A deficiency is probably much more common in patients with severe protracted jaundice than is now generally recognized. Wolbach¹⁸ showed that the ocular changes, i. e., xerosis and keratomalacia, are among the last to appear in patients and animals with vitamin A deficiency. The six patients here described showed definite histologic evidence of avitaminosis, although they were clinically without signs or symptoms of it.

Although this study was made entirely on infants, it is reasonable to assume that the conclusions that are drawn from it are applicable to adults as well. There is no reason to suppose that the mechanism of absorption of vitamin A in children is different from that in adults. Moreover, the literature contains reports of cases of adults¹⁹ with severe protracted jaundice in whom vitamin A deficiency developed in spite of the fact that they received definitely adequate diets.

¹⁴ Schmidt, W., and Schmidt, C. L. A. Univ. California Publ. Physiol. **7** 211, 1930. Greaves, J. D., and Schmidt, C. L. A. J. Biol. Chem. **105** 2221, 1934.

¹⁵ Karrer, P., Morf, R., and Schopp, K. Helvet. chim. acta **14** 1431, 1931.

¹⁶ Karrer, P., Helfenstein, A., Wehrli, H., and Wettstein, A. Helvet. chim. acta **13** 1084, 1930.

¹⁷ Mueller, J. H. J. Biol. Chem. **27** 463, 1916.

¹⁸ Blackfan and Wolbach.⁷ Wolbach and Howe^{2a, c}

¹⁹ Blegvad.⁴ Owen and Hennessey.⁶

SUMMARY

Microscopic evidence of vitamin A deficiency was found in six of eleven infants with severe protracted jaundice due to congenital atresia of the bile ducts in spite of the fact that they were receiving diets adequate in vitamin A. The deficiency occurred in five of the six infants who lived at least six months. The sixth infant to show evidence of avitaminosis was a premature twin infant.

The presence of this deficiency disease may be overlooked because the specific ocular changes of vitamin A deficiency occur late in the course of the disease.

The deficiency apparently occurs as a result of failure of absorption of vitamin A from the gastro-intestinal tract due to the absence of bile.

There is evidence indicating that the parenteral administration of vitamin A is effective in patients in whom vitamin A deficiency develops as a result of severe obstructive jaundice. The oral administration of the vitamin together with bile salts also is possibly of value.

SUSCEPTIBILITY TO DENTAL CARIES IN THE RAT

VI INFLUENCE OF ORANGE JUICE AND THE ACID-BASE BALANCE OF THE DIET

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AND

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Several theories of the etiology of dental caries are based on the assumption that susceptibility to the disease depends on or is influenced by a disturbance of the acid-base balance of the body reflected as augmented acidity or impaired neutralizing power either of the saliva or of the tooth "fluids." Among those who have advanced views of this kind are Broderick,¹ Bodecker,² Jones, Larsen and Pritchard,³ Kugelmass and King⁴ and Grove and Grove.⁵ The views of these authors coincide on some points and diverge widely on others, and the evidence adduced, based on studies of human material, is no more than suggestive. Yet a degree of general acceptance has been gained by the view, particularly, that the ash reaction of the diet may be a factor of importance in dental caries, and from the experimental point of view certain scattered observations have made it desirable to submit the question to study. Mellanby and Pattison⁶ have reported that cereals, which are acid-forming foods, may be caries-promoting agents in the human dietary, these workers do not, however, interpret this observation as supporting the acid-base theory. Hanke⁷ has stressed the value of

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1 Broderick, F W Dental Medicine, St Louis, C V Mosby Company, 1928

2 Bodecker, C F Dental Cosmos **71** 586, 1929

3 Jones, M R, Larsen, N P, and Pritchard, G P Dental Cosmos **72** 439, 574, 685 and 797, 1930

4 Kugelmass, I N, and King, T B Arch Pediat **50** 307, 1933

5 Grove, C J, and Grove, C T Dental Cosmos **76** 1029, 1934

6 Mellanby, M, and Pattison, C L Brit M J **1** 507, 1932

7 Hanke, M T Diet and Dental Health, Chicago, University of Chicago Press, 1933

large doses of orange and lemon juice in combating dental caries in children, and, although these authors also incline to a different interpretation, their findings may be regarded as favoring the views under discussion since citrus fruit juices in large amounts shift the dietary ash reaction toward alkalinity. Of immediate interest to ourselves has been the fact that the methods we have used for the production and study of experimental dental caries in rats (Rosebury, Karshan and Foley⁸) involve the feeding of diets containing as the major constituent cereals—rice or corn. We have shown that chemically identical diets which were physically altered by grinding the cereal to a fine powder are entirely without caries-producing effect, but the important question remains whether caries can be produced experimentally by means of a diet with an alkaline ash.

EXPERIMENTS

The influence of an alkaline dietary ash on the production of experimental dental caries in rats was studied by two methods, one of which also involved a direct test in the rat of the observations of Hanke that citrus fruit juices fed in

TABLE 1—*Diets Used in Experimental Study of the Production of Caries*

Component	Percentage of Component in Given Diet					
	73	74	75	76	77	78
Brown rice, 10 mesh	92	92	92	92		69
Dried lima beans, 10 mesh					92	23
White potato dextrin	8	8	8	8	8	8
Orange and lemon juice	Ad lib					
Synthetic citrate mixture 1		Ad lib				
Synthetic citrate mixture 2			Ad lib			
Water				Ad lib	Ad lib	Ad lib
Spinach leaves	3 Gm per rat per day in each diet					

large quantities are effective against caries in children. At the same time, in the expectation of a reduction of experimental caries as a result of the feeding of citrus juice, two additional control groups were instituted in order to assist the analysis of such a finding. The diets employed are described in table 1.

The juice of California oranges and lemons was expressed fresh each day, strained through muslin, centrifugated at 1,000 revolutions per minute for thirty minutes and decanted. Twelve parts of orange juice by volume were mixed with one part of lemon juice, and the mixture fed in place of water in inverted bottles supplied with glass tubes adjusted to allow free drainage on suction but to minimize leakage.

Changes in the orange and lemon juice mixture on standing—alteration of flavor and increased turbidity—were an objectionable feature unavoidable under the conditions of feeding. Supplementary tests conducted with the juice mixtures under the feeding conditions indicated, however, that these changes probably did not influence the course of the experiment. Little or no change in p_H occurred.

⁸ Rosebury, T., Karshan, M., and Foley, G. J. Dent. Research **12** 464, 1932, **13** 379, 1933, J. Am. Dent. A. **21** 1599, 1934.

Cultures in neutral dextrose broth and on neutral dextrose agar and the tomato agar of Kulp and White⁹ yielded no growth in some instances and occasional colonies of yeast and staphylococci in others, so that the change was apparently not fermentative. The animals that ingested the mixture, furthermore, although deprived of water, were equivalent in growth and survival to those on the control diet (76).

The two synthetic citrate mixtures used in diets 74 and 75 were prepared as controls for the acid-base, sugar and fluid content of the natural juices (74) and for the salt content in addition to the other factors (75). The acid-base properties of the natural juices were determined as follows:

(a) In determinations of the p_H the juice of four oranges and that of two lemons were tested separately and in 12 + 1 mixtures by means of the glass electrode with the following results: oranges, p_H 3.36 to 3.81, average 3.65, lemons, p_H 2.37 and 2.38, mixtures, p_H 3.13 to 3.45, average 3.32.

(b) In determining the organic acids a method suggested by Schlatter¹⁰ was followed. Two 10 cc samples of the juice of each of two oranges were neutralized with standard sodium hydroxide, phenolphthalein being used as indicator, and the neutral mixtures were transferred to platinum crucibles, evaporated to dryness and ashed. The ash was treated with a known excess (45 cc) of standard sulphuric acid, made up to 50 cc, and a 25 cc sample of this was neutralized with standard sodium hydroxide, phenolphthalein being used as indicator. Whole and centrifugated orange juices were found to agree closely. The method yielded the following values: titrated acid, 0.168 and 0.196 normal, with the average 0.182 normal, total organic acid, equivalent to 22.6 and 25.2 cc of 0.1 normal sulphuric acid in 10 cc, or the equivalent estimated as 0.226 and 0.252 normal citric acid. Lemons were not tested, and the higher citric acid value, 0.25 normal, was selected for use.

(c) The composition of the edible portion of oranges was given by Bronson¹¹ as follows: water, 86.9 (per cent), protein, 0.8, fat, 0.2, carbohydrate and fiber, 11.6, ash, 0.5. Myers and Croll¹² found that California oranges contained 14.18 per cent sugar, made up of 8.1 per cent sucrose and 6.08 per cent reducing sugar. The latter values were used, the reducing sugar made up equally of dextrose and fructose.

(d) Bronson gave the percentage salt composition of the ash of California oranges as follows: potassium oxide, 48.94, sodium oxide, 2.50, calcium oxide, 22.71, magnesium oxide, 5.34, ferric oxide + aluminum oxide, 0.97, manganese oxide (Mn_2O_3), 0.37, phosphorus pentoxide, 12.37, sulphur trioxide, 5.25, silicon dioxide, 0.65, chlorine, 0.92. Since potassium is the predominating cation, synthetic mixture 1 (without added salts) was prepared as a simple citric acid and potassium citrate buffer with sugar added. Using the values derived, 50 cc samples of 0.25 normal citric acid were titrated electrometrically with 5 times normal potassium hydroxide, 0.82 cc of the latter was found required to produce p_H 3.3, this is equivalent to 4.53 Gm of potassium hydroxide per liter of 0.25 normal citric acid. For mixture 2 we used 3 Gm of potassium hydroxide per liter of

9 Kulp, W. L., and White, V. *Science* **76** 17, 1932.

10 Schlatter, H., in Allen, A. H. *Commercial Organic Analysis*, ed. 5, Philadelphia, P. Blakiston's Son & Co., 1923, vol. 1, p. 749.

11 Bronson, B. S. *Nutrition and Food Chemistry*, New York, John Wiley & Sons, Inc., 1930.

12 Myers, V. C., and Croll, H. M. *J. Biol. Chem.* **46** 537, 1921.

0.25 normal citric acid as calculated from the data on the ash, and the remaining salt values were calculated to correspond. Iron, aluminum and silicon were omitted. A separate salt mixture was prepared as follows from chemically pure ingredients, and 20 cc added as a component of each liter of mixture 2.

Sodium chloride	7.6 Gm
Sodium sulphate	19.3 Gm
Magnesium sulphate	23.2 Gm
Magnesium carbonate (basic $3\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 3\text{H}_2\text{O}$)	43.7 Gm
Tricalcium phosphate	134.5 Gm
Calcium carbonate	72.5 Gm
Distilled water to make	1,000 cc

The two synthetic mixtures were prepared as follows:

	1 (Without Salts)	2 (With Salts)
Citric acid (M. W. 210.08)	21.7 Gm	21.7 Gm
Potassium hydroxide, electrolytic sticks	4.53 Gm	3.0 Gm
Salt mixture (as in the foregoing formula)		20.0 cc
Sucrose	81.0 Gm	81.0 Gm
Dextrose	30.4 Gm	30.4 Gm
Fructose	30.4 Gm	30.4 Gm
Distilled water to make	1,000 cc	1,000 cc

Both mixtures were somewhat flat but not objectionable in taste and were well tolerated by the animals. They were prepared fresh at intervals of about a week, and the stock was kept in the refrigerator between feedings. They did not undergo detectable change on standing during these intervals. Several samples were tested for p_{H} by the glass electrode at intervals during the course of the experiment. The results ranged from p_{H} 3.25 to p_{H} 3.39, with the two mixtures in close agreement.

Dried lima beans were selected for use in diets 77 and 78 because they are somewhat similar both physically and chemically to rice, but instead of the slightly acid reaction of rice ash their ash has a strongly alkaline reaction, due chiefly to their higher content of potassium and sodium. The ash reactions of the dietary ingredients and of the diets as fed are given in the next paragraph. Diet 77 was not successful in that animals that ingested it did not survive more than twenty-two days of experimental feeding. The addition of ammonium chloride as 1, 1.5 and 2 per cent of the diet, respectively, with three successive groups of animals did not prolong survival. The results with this diet are consequently omitted from the tables but are mentioned in the body of the report.

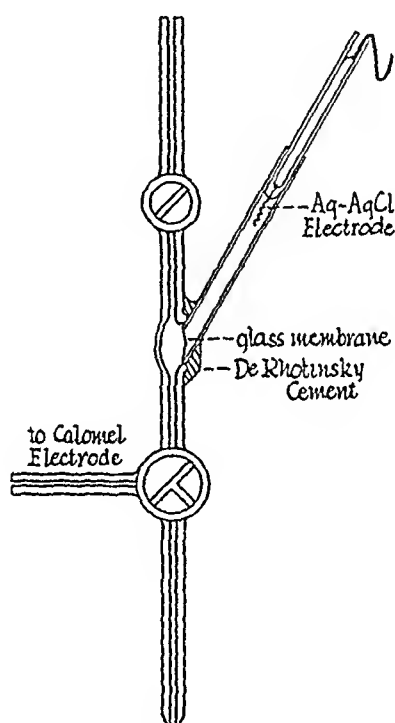
The food and fluid intakes were measured at intervals in all groups except 77. The food intake averaged 52 Gm of dry diet per rat per day in all groups, and the fluid intake 64 cc per rat per day, with no characteristic group differences. The intake of spinach per rat per day is estimated as 2 Gm of the edible portion. To calculate the ash reactions of the diet as fed, the following values for dietary ingredients, given as cubic centimeters of normal acid or base per hundred grams, were used: rice, 9 acid (Sherman¹³), lima beans, 41.65 base (Sherman and Gettler¹⁴), orange juice and synthetic citrate 2, 8.6 base, and synthetic citrate 1, 8.1 base (both calculated from the data given), and spinach, 21.8 base (calculated from Sherman's data). These values yield the following for the ash reactions of the diets as fed in terms of cubic centimeters of normal acid or alkali per day: diets 73 and 75, 0.55 base, diet 74, 0.53 base, diet 76 (control), 0.01

13 Sherman, H. C. *Chemistry of Food and Nutrition*, New York, The Macmillan Company, 1928.

14 Sherman, H. C., and Gettler, A. D. *J. Biol. Chem.* **11**: 323, 1912.

base, and diet 78, 0.65 base. The control thus appears to have been approximately neutral as fed, the acid effect of the rice having been counterbalanced by the spinach ingested, and all the experimental diets were definitely alkaline.

In an attempt to check these calculated values determinations were made during the course of the experiments on the p_H of the urine and the p_H and the carbon dioxide content of the blood plasma. Blood for these purposes was drawn by cardiac puncture under liquid petrolatum and transferred to oxalate tubes containing oil. These were centrifugated under solid paraffin. Urine was collected overnight by keeping the animal in an improvised metabolism cage consisting of a 10 pound (4.5 Kg) tin with the bottom removed, set over a circle of 10 mesh wire gauze into a large glass funnel which had another circle of gauze placed at a lower level and sealed to the glass with paraffin. The stem of the funnel was connected by means of a rubber stopper with a separatory funnel below. The apparatus was



Glass electrode chamber for anaerobic determination of p_H of blood and urine

filled with liquid petrolatum up to about the lower third of the tapered portion of the large funnel and about 1 cc of toluene added. Animals were placed in groups of two in the tin in the evening, and the urine was removed and tested the following morning. The urine thus obtained was clear and normal in color and odor. All p_H determinations were made at 25 C on the glass electrode by means of the vessel shown in figure 1. The plasma and urine samples under oil were allowed to stand in the constant temperature chamber (F Rosebury¹⁵) for about fifteen minutes and were then aspirated into the vessel and the stopcocks closed. The vessel was washed between determinations by flushing it with 95 per cent alcohol and then with distilled water. By this procedure good checks were obtained with standard buffers. The values for p_H as given have been corrected to 37 C by

¹⁵ Rosebury, F. J. Indust. Engin. Chem. (Anal. Ed.) 4: 398, 1932.

the method of Cullen¹⁶ Determinations of the carbon dioxide content were made on 0.2 cc of plasma by the method of Van Slyke and Neill¹⁷

The additional methods employed were the same as those previously used and described The jaws and teeth of one lateral half of the mandible of each animal were sectioned and examined microscopically, the results for caries recorded by the use of the index value and the indexes compared by the statistical method outlined in the preceding report in this series¹⁸

TABLE 2—*The Incidence of Caries and the Average Values for Plasma p_H and for Carbon Dioxide and Urine p_H in Groups 73 to 78*

Group	Animals	Additions to Basal Diet	Experimental Period, Days	Caries		Plasma		
				Animals, per Cent	Index	p_H	CO ₂ , Vol per Cent	Urine, p_H
73	12	Orange lemon juice ad lib, no water	50-123	100	11.3 \pm 1.1	7.30	61.2	6.80
74	11	Citrate mixture 1 ad lib, no water (without salts)	50-130	100	10.2 \pm 1.5	7.30	68.0	6.99
75	12	Citrate mixture 2 ad lib, no water (with salts)	50-130	100	7.3 \pm 1.4	7.33	66.9	7.09
76	10	None (control)	50-116	90	7.6 \pm 1.8	7.34	65.8	6.80
78	11	Dried Lima beans McCullum stock diet (5 animals)	50-130	81	6.9 \pm 1.8	7.27 7.29	69.2 67.4	6.80 6.75

TABLE 3—*Significance of the Effects on the Incidence of Caries of the Addition to the Basal Deficient Diet Containing 10 Mesh Rice, Dextrin and Spmach, of Orange and Lemon Juice, Citrate Mixtures, Dried Lima Beans and Sugars Other Than Dextrin*

Group	Animals	Caries Index m*	mo	D	$\frac{D}{\sigma_D}$	Odds Against Random Occurrence of Difference
Pooled control	94	7.6 \pm 0.6				
73	12	11.3 \pm 1.1	8.0 \pm 0.5	3.3 \pm 1.2	2.8	195 to 1
74	11	10.2 \pm 1.5	7.9 \pm 0.5	2.3 \pm 1.6	1.4	5 to 1
75	12	7.3 \pm 1.4	7.6 \pm 0.5	0.3 \pm 1.5	0.2	<1 to 1
78	11	6.9 \pm 1.8	7.5 \pm 0.5	0.6 \pm 1.9	0.3	<1 to 1
73, 74, 75	35	9.5 \pm 0.8	8.1 \pm 0.5	1.4 \pm 0.9	1.6	8 to 1
30, 31	10	11.6 \pm 1.3	8.0 \pm 0.5	3.6 \pm 1.4	2.6	106 to 1

* m, mo, means, D, difference between two means, σ_D , standard deviation of D, values following \pm signs are standard deviations The deviation of these values and the method of analysis are described in the preceding paper in this series¹³

RESULTS

Data on the incidence of caries, the p_H and the carbon dioxide determinations are given in table 2, and the significance of the results, in table 3 The values for plasma p_H and carbon dioxide and for urine p_H are within the normal range, as indicated by comparison with the values for animals on the modified McCullum stock diet The calcification of

16 Cullen, G. E. J. Biol. Chem. **52**: 501, 1922

17 Van Slyke, D. D., and Neill, J. M. J. Biol. Chem. **61**: 523, 1924

18 Rosebury, T., and Karshan, M. Arch. Path. **20**: 697, 1935

the teeth and bones of these animals, not recorded in the tables, was uniformly poor, and the separate groups were not mutually distinguishable on this basis. The levels and ratios of calcium and phosphorus in these diets are given in table 4.

The results as indicated in tables 2 and 3 were as follows. No significant reduction in the incidence of caries was obtained in any of the experimental groups as compared with the control or with the pooled control group used for more precise statistical analysis. Thus the reduction of caries expected in the group that received natural citrus juice was not obtained, instead the incidence of caries was increased in this group to an extent bordering on statistical significance. The three groups that received orange and lemon juice or a citrate mixture showed decreasing caries indexes from the group that received natural juice, with the highest index, to the group that received the citrate mixture plus salts, with the lowest index, but it is doubtful whether this trend is real or merely the result of random variation. These questions are discussed later in this paper.

TABLE 4—*Levels and Ratios of Calcium and Phosphorus in Diets*

	Diet				
	73	74	75	76	78
Calcium	0.044	0.029	0.044	0.029	0.042
Phosphorus	0.255	0.247	0.255	0.247	0.267
Ratio	0.17	0.12	0.17	0.12	0.16

It has been noted that diet 77, in which ground rice was replaced entirely by dried lima beans ground to pass a 10 mesh sieve, did not allow survival for the period usually required with rice diets to produce the first fissure-caries lesions. In twenty-eight animals placed on the three modifications of this diet the period of survival ranged from three days to only twenty-two days. The jaws of all of these animals were nevertheless sectioned and examined microscopically, and it seems worthy of note that one of these animals, which had survived only seven days of experimental feeding, showed a characteristic fissure-caries lesion of stage 3 in the second molar. The third molar had not yet erupted. Over the lesion, impacted in the fissure, was a particle recognizable as lima bean by comparison with sections of beans made in different planes and distinct from rice and spinach sectioned similarly.

COMMENT

It is clear from these results that the feeding of orange and lemon juice or synthetic mixtures similar in chemical composition but lacking protein, fat and vitamins exerts no protective action against dental caries.

produced in rats with a coarse rice diet. With the natural juice and the synthetic mixture lacking added salts the incidence of caries was increased. If this increase is significant, it may be related to that produced in a previous experiment⁸ when dextrose or saccharose was substituted for the dextrin in the basal diet (diets 30 and 31, table 3) and may depend on the added sugar in the diet. It is especially worthy of note in this connection that Shibata, as recently determined by Cox¹⁹ from a direct translation of the original Japanese paper, reported that dextrose and saccharose were more effective in producing caries than dextrin. We had previously misinterpreted this statement in the English version of Shibata's report. These observations, augmented by the recent findings of Koehne, Bunting and Morrell²⁰ in children, suggest that the importance of different sugars in the caries-producing diet is worth further investigation. Additional studies on this phase of the subject are now in progress in this laboratory.

The result obtained with diet 78, which contained dried lima beans in addition to rice, both ground to pass a 10 mesh sieve, is interesting from two points of view. It appears, first, that in the absence of added sugar, which may have accelerated caries in diets 73 to 75, the incidence of caries was not altered with a diet having a definitely alkaline ash. The implication is clear that the reaction of the ash of the diet cannot be regarded as a factor in the production of experimental dental caries in rats. Furthermore, it appears likely, although not satisfactorily demonstrated by these results, that lima beans may have caries-producing properties similar to those of rice, as suggested by the fact that the incidence of caries in group 78 was not reduced despite reduction in the dietary proportion of rice, and by the finding of caries in a single instance on the rice-free diet 77.

SUMMARY AND CONCLUSIONS

Eighty-four rats 22 days of age were distributed among groups that received, respectively, the basal caries-producing diet containing 10 mesh rice, dextrin and spinach, and the following modifications of it: the basal diet with orange and lemon juice (12 + 1) fed ad libitum in place of water, the basal diet with a synthetic citrate mixture having the acid-base properties and sugar content of the natural citrus juice mixture, the basal diet with a synthetic citrate mixture having the acid-base properties and sugar and salt content of the natural juice, a diet in which all of the rice was replaced by 10 mesh dried lima beans, and a diet in which part of the rice was replaced by lima beans. The method

19 Cox, C. J. Personal communication, 1934.

20 Koehne, M., Bunting, R. W., and Morrell, E. *Am. J. Dis. Child.* **48**: 6, 1934.

of preparing the citrate mixtures has been described. The acid-base properties of the diets were calculated and the animals tested for the p_H and carbon dioxide content of the plasma and the p_H of the urine. The jaws and teeth of all the animals were studied microscopically for caries. The results appear to warrant the following conclusions:

The feeding of orange and lemon juice *ad libitum* in place of water with a caries-producing diet of 10 mesh rice did not reduce the incidence of caries but resulted in an increase of doubtful significance. This result was duplicated to less degree when the natural citrus juice was replaced by synthetic citrate mixtures having in one case the acid-base properties and sugar content and in the other the acid-base properties, sugar content and mineral content, of the natural juice. The increase of caries obtained, if significant, may depend on the fermentable sugar present in these mixtures.

A diet in which enough of the rice was replaced by dried lima beans to produce a mixture with a definitely alkaline ash did not yield a significantly lower incidence of caries than was obtained with the basal rice diet, which had an approximately neutral reaction *as fed*. A rice-free lima bean diet did not allow survival beyond twenty-two days of feeding but produced a typical lesion of fissure-carious in a rat that survived only seven days of experimental feeding.

RENAL LESIONS FOLLOWING INJECTION OF SODIUM DEHYDROCHOLATE IN ANIMALS WITH AND WITHOUT BILIARY STASIS

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AND

ABRAHAM CANTAROW, M D

PHILADELPHIA

In the course of our studies of biliary stasis and decompression in the cat¹ sodium dehydrocholate² was administered in order to investigate the effect of a bile salt of supposedly extremely low toxicity on certain hepatic lesions developing under those experimental conditions. To our surprise, a severe and rather characteristic lesion was uniformly found in the kidneys in animals with and without biliary stasis. Similar changes were found in dogs without stasis. These observations are of interest in connection with the controversial problem of epithelial regeneration in the kidney and in regard to the probable mechanism of diuresis which has been observed clinically following the administration of sodium dehydrocholate.

MATERIAL AND METHODS

Fifty-one cats (weighing from 1.3 to 4.3 Kg) and four dogs (weighing from 6 to 15 Kg) were employed in this study. They were maintained on a diet of fresh raw scrap meat and milk. Five series of experiments were performed, as follows:

EXPERIMENT 1—Sodium dehydrocholate, in a 20 per cent solution, was injected into a vein in the leg in sixteen cats at twenty-four hour intervals over periods of from one to seventeen days, in individual doses of 0.2 cc per kilogram of body weight. This dose would not be regarded as excessive for clinical use. The animals were killed by bleeding under light ether anesthesia twenty-four hours after the last previous injection of sodium dehydrocholate. Determinations of the

This investigation was aided by the Jefferson Medical College Hospital Tumor Clinic and the Martin Research Fund.

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1 (a) Cantarow, A, and Stewart, H L. *Am J Path* **11** 561, 1935. (b) Stewart, H L, and Cantarow, A. *Am J Digest Dis & Nutrition* **2** 101, 1935. (c) Stewart, H L, Cantarow, A, and Morgan, D R. *Arch Path* **19** 807, 1935. (d) Stewart, H L, and Lieber, M M. *ibid* **19** 34, 1935. (e) Cantarow, A, Stewart, H L, and Lieber, M M. *ibid* **20** 535, 1935.

2 Sodium dehydrocholate is available in the form of decholin sodium (obtainable from Riedel-de Haen, Inc, New York, which has been accepted by the Council on Pharmacy and Chemistry of the American Medical Association). The decholin employed in the present study was supplied by Mr Paul de Haen.

nonprotein nitrogen were made on blood obtained at this time, and urine aspirated from the bladder was submitted to routine chemical and microscopic examination

EXPERIMENT 2—In three cats sodium dehydrocholate (0.2 cc per kilogram of body weight, in a 20 per cent solution) was injected into a vein in the leg. The animals were killed by bleeding under light ether anesthesia two, three and four days, respectively, after injection. Specimens of blood and urine were examined as in experiment 1.

EXPERIMENT 3—In three cats sodium dehydrocholate (0.2 cc per kilogram of body weight, in a 20 per cent solution) was injected into the portal vein, which was exposed at laparotomy under ether anesthesia. The animals were killed by bleeding under light ether anesthesia one, two and three days, respectively, after injection. Specimens of blood and urine were obtained and examined as in experiment 1.

EXPERIMENT 4—Sodium dehydrocholate (0.2 cc per kilogram of body weight, in a 20 per cent solution) was injected daily into a vein in the leg in four dogs, which were killed by bleeding under light ether anesthesia twenty-four hours after one, two, three and four injections, respectively. Blood was obtained daily from the heart for determinations of the nonprotein nitrogen. No urine was present in the bladder in any case at autopsy.

EXPERIMENT 5—In twenty-nine cats the common bile duct was ligated close to the duodenum at laparotomy under ether anesthesia. After twenty-four hours of biliary stasis sodium dehydrocholate (0.2 cc per kilogram of body weight, in a 20 per cent solution) was injected into a vein in the leg. Individual animals received from one to fifteen injections during periods of stasis of from two to eighteen days, with the exception of a few instances of prolonged stasis in which a few injections were made at forty-eight hour intervals, they were given at intervals of twenty-four hours. The animals were killed by bleeding under light ether anesthesia twenty-four hours after the last previous injection. Specimens of blood were obtained and examined as in experiment 1. All cases were excluded from present consideration in which there was any evidence of infection or of patency or reconstruction of the common duct or entrance of bile into the intestine through anomalous or accessory ducts.

Pieces of tissue were fixed in a dilute solution of formaldehyde U. S. P. (1:10) or solution of formaldehyde with Zenker's solution. Some were subsequently frozen, cut and stained with Nile blue sulphate and scarlet red, the remainder being blocked in paraffin, cut and stained with hematoxylin and eosin and Mallory's connective tissue stain.

EXPERIMENT 1

Observations were made on sixteen cats killed twenty-four hours after from one to fifteen injections into a vein in the leg.

Gross Observations—The kidneys were swollen to a moderate degree after from one to eight injections but were either normal in size or smaller than normal in animals receiving a greater number of injections. The surface, which was rather uniformly yellow in the early stages, tended to be more definitely brown in the later stages. The cut surface was consistently edematous, and the tissue was soft in consistency. The cortex was relatively narrow, even in kidneys that were increased in size, this change becoming progressively more marked with increasing numbers of injections. In the later stages, the thickness of the cortex was diminished by from 30 to 40 per cent. In the shorter experiments (from one to eight injections) the outer zone of the medulla frequently showed fine bright red punc-

tate and linear markings, later, fine gray striations were occasionally noted in this situation

Microscopic Observations—By far the most outstanding histologic changes occurred in the tubules, which showed variable degrees of dilatation and degeneration and evidences of regeneration of the lining epithelium. Relatively minor changes were noted in the interstitial tissue of the outer medullary and inner cortical zones, which showed evidence of a mild inflammatory reaction, with vascular congestion, edema, slight infiltration by cells and, in the later stages, increase in connective tissue. Glomerular lesions were inconstant and relatively inconspicuous and in no instance resembled those characteristic of glomerulonephritis.

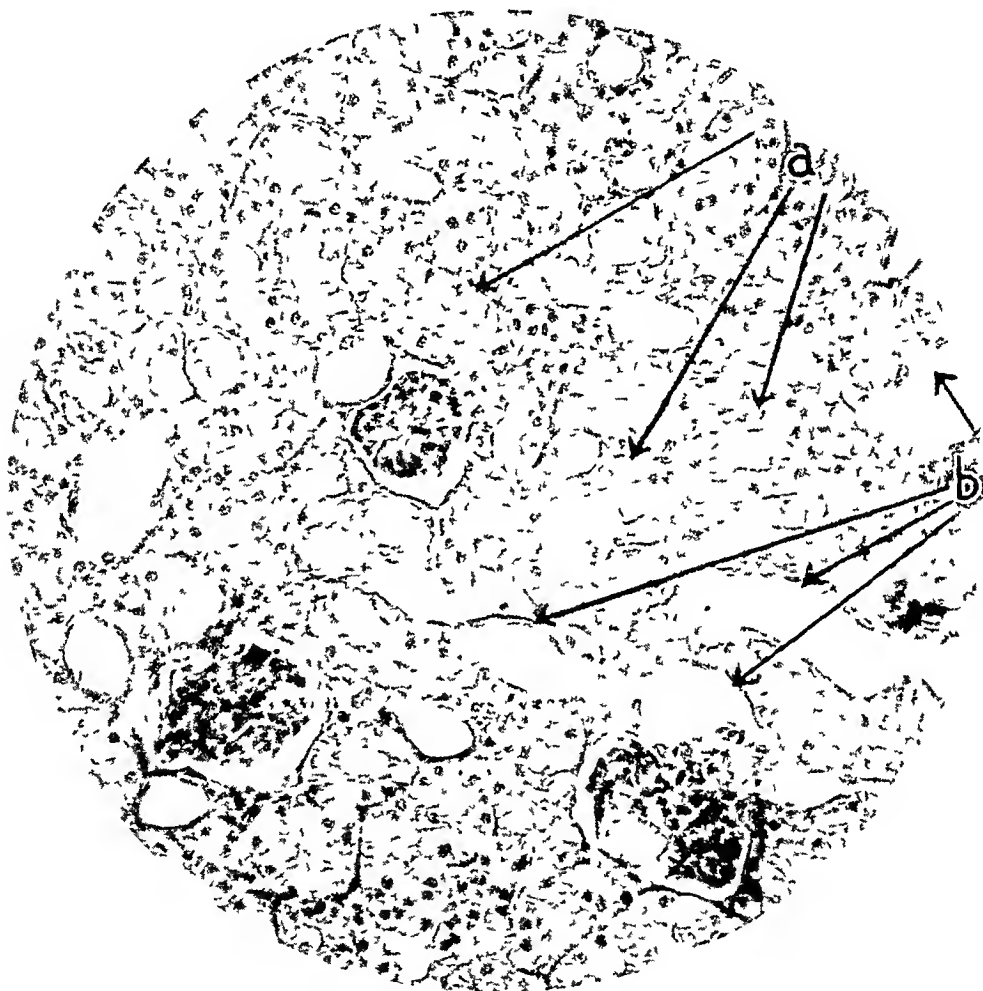


Fig 1—Section of kidney showing (a) tubules with increased outside diameter and (b) tubules with increase in the diameter of the lumen associated with flattening of the lining cells. Many cells show hydropic degeneration and necrosis. Magnification, about 200 \times .

Dilatation of Tubules Some increase in the outside diameter of the tubules was noted throughout the entire course of the experiment. This was frequently diffuse but in some instances was particularly marked in localized portions of the tubule. This dilatation was prominent after a single injection and persisted as a prominent feature in animals receiving seven or eight injections, decreasing subse-

quently but still being present to a variable degree. The diameter of the lumen was also increased, this increase being proportional both to the increase in outside diameter and to the diminution in the height of the lining cells, described in the next section (figs 1 and 2).

Regressive Changes in Tubular Epithelium Marked regressive changes were present in the tubular epithelium in every case. The intensity of these changes did not increase in proportion to the number of injections, but rather appeared to be related to the nature of the epithelial cell predominating in each case, i. e., there was a variable degree of susceptibility of the various forms of regenerated

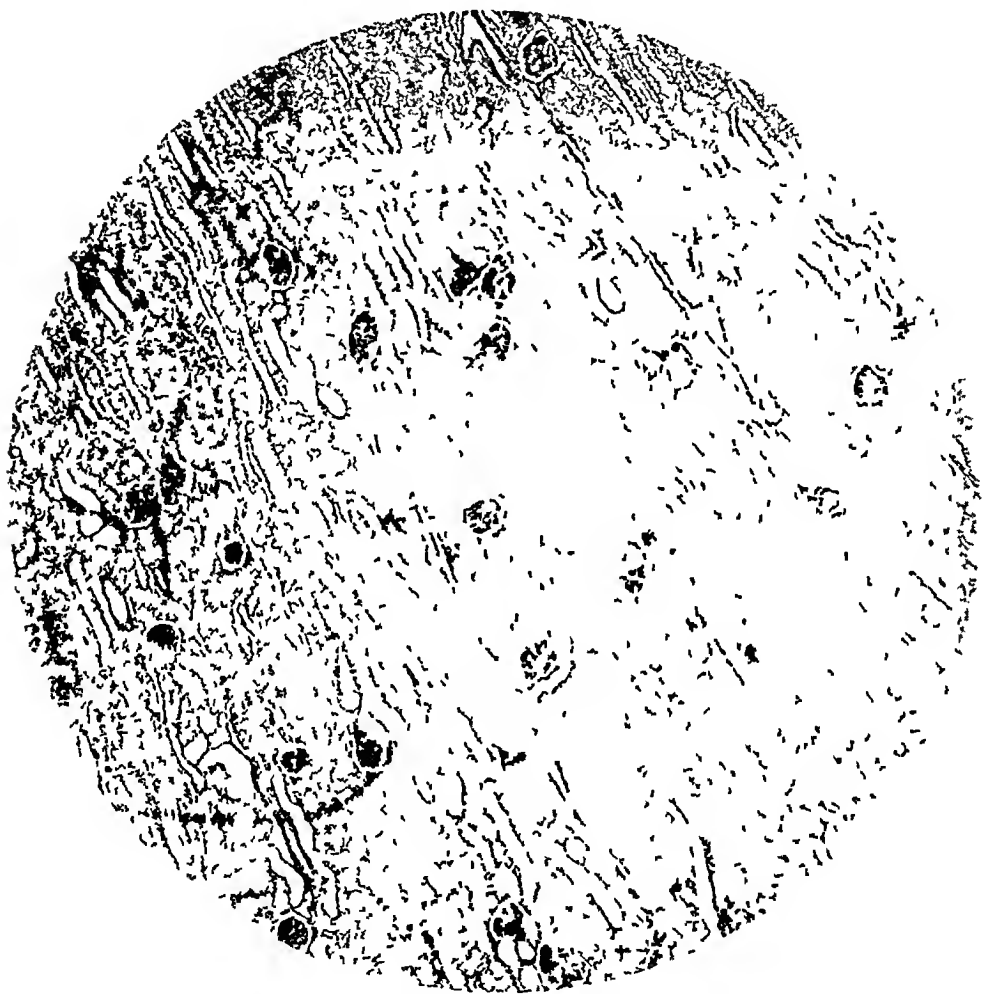


Fig 2—Section of kidney showing extensive dilatation of tubules, flattening of epithelial cells and pale, reticular character of certain cells lining straight tubules. Magnification, about 50 \times

cells to subsequent injury. Some degree of regressive change, however, was observed in different instances both in atypical regenerated and in normal-appearing cells. In many cases, particularly in the early stages, the process was generalized, but in some instances following several injections it appeared to be more intense in the straight tubules of the inner cortical and outer medullary zones.

After a single injection there were extensive degeneration and necrosis of the cells that could be recognized as apparently the original tubular epithelial cells.

In the early stage of the degenerative process the cell was swollen and was either homogeneous and acidophilic or hydropic, with pale, granular and reticulated cytoplasm and curvilinear striations, the long axis of the cell being tilted in relation to the basement membrane (figs 1 and 2). As the regressive process progressed the nuclei showed the characteristic evidences of necrosis, the cellular outlines became obliterated and cytoplasmic disintegration, beginning at the free border, progressed inward, leaving a ragged luminal outline and, in occasional tubules, a completely denuded basement membrane. Degenerative changes were most extensive after one or two injections, involving particularly the original lining cells of the tubules. With increasing numbers of injections (from five to eight) the predominating type of epithelium was of the atypical, flattened form described later (under regeneration) which, although apparently resistant to subsequent injury, nevertheless was involved in the regressive process to a variable extent in certain cases. In the late stages (twelve or more injections) a large proportion of the tubules were lined by tall columnar cells which resembled the original cells, and in these the intensity of degeneration and necrosis sometimes approached that observed after a few injections.

The lumens of the tubules contained shreds of lining membrane, hyaline droplets, albuminous material and casts, and in some instances they were filled with cells, desquamated singly or *en masse*. Calcific deposits were frequently observed within the lumens of the tubules and at times in necrotic lining cells in both the cortex and the medulla.

Regeneration of Tubular Epithelium. Evidences of regeneration were obtained in every instance in all stages of the experimental period. This was characterized particularly by the development of an atypical form of regenerated cell, which was present in all cases, and in some instances in great numbers, after a single injection. These atypical cells, when first formed, were exceedingly flat when viewed in cross-section, their nuclei were two or three times the normal size, they were usually oval but sometimes round, the long diameter being sometimes vertical, sometimes parallel and sometimes situated obliquely to the basement membrane. The relatively large size of the nuclei in proportion to the cytoplasm caused them to protrude into the lumen of the tubules, producing a beadlike appearance. When cut tangentially, these cells appeared large, oval or round, sometimes irregular, with a slightly basophilic cytoplasm and a pale nucleus containing numerous fine particles of chromatin, one or more dense masses of chromatin and sometimes vacuoles (fig 3). These flat cells were observed in all portions of the renal tubule in different animals. In the early period of the experiment (from one to three injections) they tended to occur in groups of tubules in triangular areas in the outer layer of the cortex, the base lying toward the surface of the kidney. In the later period they were more diffusely distributed, but involved particularly the tubules in the inner cortical and outer medullary zones. This markedly flattened cell was most numerous in animals receiving from four to seven injections, the great majority of the tubules in some cases being lined by this type of epithelium. In the later stages these cells usually diminished in number.

Other atypical forms of epithelial cells were present throughout the course of the experiment. These appeared to represent transition stages between the very flat cells already described and the mature tubular epithelium. They varied in height from low cuboidal to tall columnar types, the cytoplasm becoming less basophilic as the cell increased in height. The nuclei became smaller and rounder as the height of the cell increased, the arrangement of chromatin simultaneously changing from that noted in the case of the flat cells to one approaching normal,

with a distinct tendency toward hyperchromasia. In many instances single tubules were lined by flat, cuboidal and columnar cells, the nuclei of which were situated at different levels in relation to the basement membrane, imparting an extremely irregular appearance to the epithelial lining. Syncytial masses of cells possessing all the characteristics of viable newly regenerated cells, as already described, were observed, particularly in the tubules of the inner layer of the cortex and the outer layer of the medulla, these at times practically occluded the lumen of the tubule. These cells could be readily differentiated from degenerated and necrotic masses of cells desquamated into the lumen.

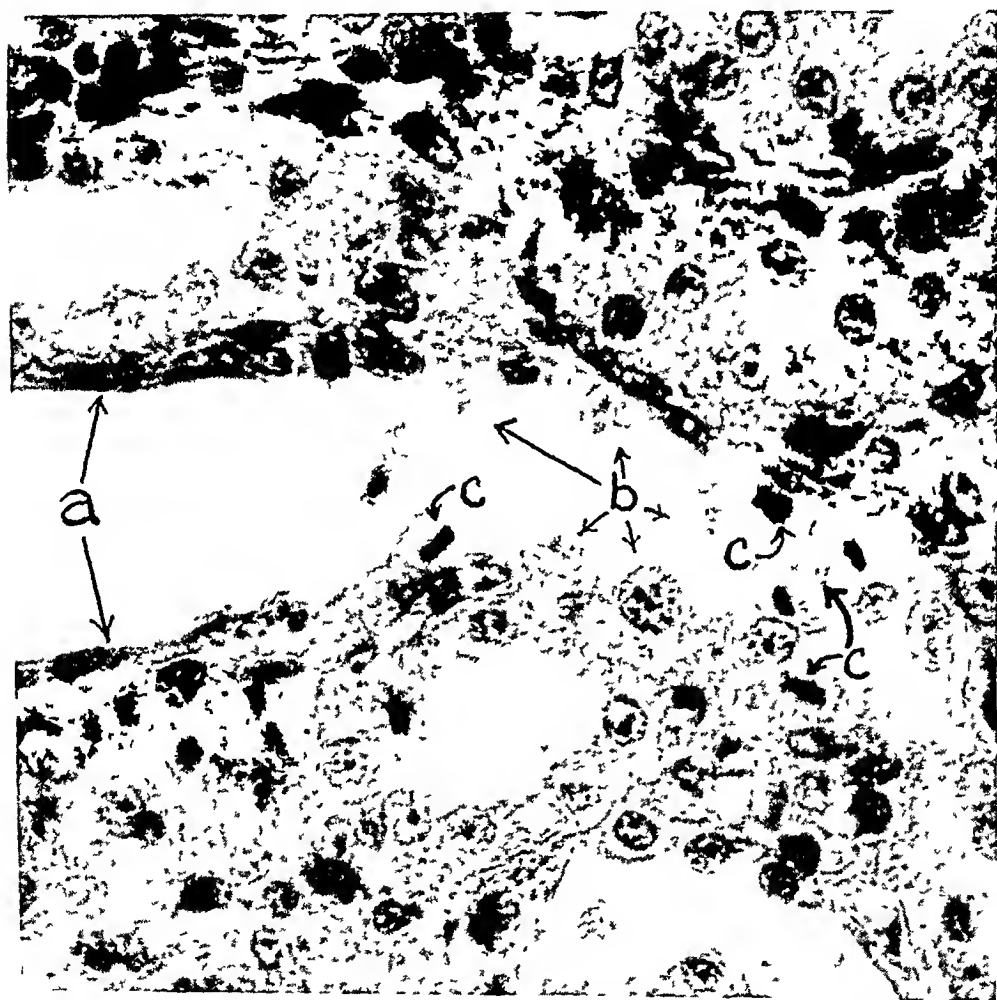


Fig 3—Section of kidney, with tubule crossing field from left to right, showing flattened type of atypical regenerated cell (a) in cross-section and (b) cut tangentially and (c) four mitotic figures in the same plane. Magnification, about 750 \times .

Mitotic figures were observed in association with all varieties of epithelial cells, both atypical and normal, in all portions of the tubule, being most consistently present and most numerous in the tubules of the inner cortical and outer medullary zones (fig 3). They were noted at every stage of the experiment, although not in every animal, being most numerous after from two to seven injections and decreasing subsequently except in one case in which large numbers were seen.

after twelve injections. In some places these dividing cells appeared to be growing beneath the degenerated cells of the original lining membrane, but at times they projected into the lumen of the tubule. In some cases cells undergoing mitosis were scattered singly throughout the renal tissue, while in others several such cells were present in a single tubule.

Interstitial Tissue Changes in the interstitial tissue were limited particularly to the outer layer of the medulla and the inner layer of the cortex, being less constant and less marked in the latter situation. These changes were apparently inflammatory, consisting of capillary congestion and thrombosis, edema, cellular infiltration and fine fibrosis of the interstitial tissue. No fibrin was observed in any instance. After a single injection the intertubular capillaries in these situations were dilated, hyperemic and occasionally thrombosed and contained an increased number of polymorphonuclear leukocytes. Few or no inflammatory cells were noted in the interstitial tissue during this period. After two or more injections edema, cellular infiltration and a fine fibrosis developed, these changes being distributed generally throughout the region of the corticomedullary junction, with focal areas of increased involvement, particularly in the outer layer of the medulla. Edema was most marked after from six to eight injections and in some instances was so extensive as to cause compression of the surrounding tubules. Fibrosis was never extensive, but cellular infiltration was marked after from fourteen to sixteen injections, the predominating cells being mononuclear leukocytes and lymphocytes. In a few cases polymorphonuclear and mononuclear leukocytes were observed in the lumens of the tubules in this region.

Glomeruli Morphologic evidence of glomerular damage was absent in most cases and when present was slight and inconstant. There were variable changes in size, cellularity and congestion of glomerular tufts in different cases as well as in different portions of the kidney in individual animals. These changes bore no consistent relation to the number of injections or to the degree of involvement of the remainder of the kidney. The capsular spaces were at times dilated and at times small and frequently contained acidophilic granular material, hyaline droplets and desquamated cells. The parietal cells were often desquamated, and the capsule was frequently lined by pale, flat or cuboidal basophilic cells which resembled the newly regenerated cells of the renal tubules. At times these cells were arranged in syncytial masses and were occasionally observed in mitosis. In a few animals in the late stages an occasional glomerulus in the deep layer of the cortex was involved in the inflammatory and fibrotic process occurring in this area.

Stainable Lipid The distribution of stainable lipid in fourteen nonpregnant adult animals was as follows: (1) convoluted tubules only, two cases, (2) convoluted and Henle tubules, seven cases, (3) convoluted, Henle and collecting tubules, five cases. Fatty casts were present in large numbers in two cases, and in one instance remnants of the old necrotic, desquamated lining membrane within the lumens contained numerous droplets of lipid, while the atypical regenerated lining cells of the same tubules contained no stainable lipid. Stainable lipid was found in the extremely flat variety of regenerated epithelium in only one case, but it was occasionally present in transitional forms. In several instances a large proportion of the convoluted tubules contained no stainable lipid.

Blood and Urine—We have obtained values for the nonprotein nitrogen of the blood as high as 55 mg per hundred cubic centimeters in apparently normal cats. In five cats, twenty-four hours after a single injection of sodium dehydrocholate the values for the nonprotein nitrogen of the blood were as follows: 30, 46, 114, 127

and 148 mg per hundred cubic centimeters, the corresponding values for creatinine being 14, 17, 54, 32 and 26 mg, respectively. In ten animals receiving from two to fifteen injections the concentration of the nonprotein nitrogen ranged from 30 to 67 mg, being below 55 in six and above 55 in four instances. The level of the nonprotein nitrogen of the blood bore no demonstrable relation to the number of injections after the first injection.

The urinary findings were extremely variable. Several specimens were apparently normal, others contained from a trace to a heavy cloud of albumin, from a few to many hyaline and granular casts per low power field and from 15 to 60 pus cells per low power field.

EXPERIMENT 2

Observations were made on three cats killed two, three and four days following a single injection into a vein in the leg.

Grossly, the kidneys resembled those described previously. In the cat killed two days after the injection there were moderate edema, degeneration and necrosis of the tubular epithelium, with marked desquamation of the lining cells and occasional deposits of calcium. Regeneration of atypical flattened cells was relatively slight, and mitotic figures were occasionally seen. A few casts were seen in the tubules, and there was evidence of a mild inflammatory reaction in the region of the corticomedullary junction. Dilatation of the tubules and regressive changes in the tubular epithelium were less marked than in animals studied twenty-four hours after a single injection, as described previously.

In the cat killed three days after a single injection there was marked, diffuse dilatation of the tubules, about 35 per cent of which were lined by the flattened, atypical variety of regenerated epithelium, chiefly cuboidal. There was a moderate degree of degeneration, some calcification and marked edema of the tubular epithelial cells. Many mitotic figures were observed. There was a mild inflammatory reaction in the outer medullary interstitial tissue.

In the cat killed four days after a single injection all regressive changes were distinctly less severe than in the cat killed three days after the injection. There was but slight dilatation of the tubules of the cortex and outer layer of the medulla, many of which were lined by cells which, although not markedly flattened, could be readily recognized as newly regenerated cells, apparently approaching normal in size, shape, staining reaction and nuclear characteristics. In places these cells were arranged in syncytial masses which occupied the entire lumen of the tubule. No mitotic figures were observed, and there was little if any evidence of inflammatory reaction in the interstitial tissue.

Stainable lipids were present in the epithelium of both the Henle and the convoluted tubules in each case. In the cat studied three days after the injection fine granules of lipid were observed in the very flat and cuboidal varieties of atypical regenerated epithelium.

The concentrations of the nonprotein nitrogen of the blood two, three and four days after a single injection of sodium dehydrocholate were 46, 59 and 52 mg per hundred cubic centimeters, respectively. The urine of the cat killed after two days was essentially normal, that of the cats killed on the third and fourth days contained a light cloud of albumin and an occasional hyaline and granular cast and from 15 to 20 pus cells per low power field.

EXPERIMENT 3

Observations were made on three cats killed one, two and three days after a single injection into the portal vein.

In the cat killed one day after injection there was moderate dilatation of the tubules, with marked degeneration, necrosis and desquamation of the lining cells. There was a moderate degree of regeneration of the atypical, flattened variety of epithelium, and no mitotic figures were seen. The tubules contained numerous casts, and the inflammatory reaction in the interstitial tissue was of mild degree. These changes closely resembled those observed in animals studied twenty-four hours after a single injection into a vein in the leg.

Two days after the injection practically all the tubules showed an extreme grade of dilatation, about one third of the lining cells being of the atypical, flattened variety. Large numbers of mitotic figures were present. Regressive changes were less severe than in the cat killed one day after the injection. A few areas of calcium deposition were observed, and there was a mild inflammatory reaction in the interstitial tissue of the outer layer of the medulla.

In the cat killed three days after injection, dilatation and degeneration were distinctly less marked than in the cat killed two days after the injection. The number of atypical, regenerated epithelial cells was approximately the same, but the majority of these were cuboidal rather than markedly flattened and were definitely more normal in appearance. No mitotic figures were seen.

Stainable lipids were present in the epithelium of both the convoluted and the Henle tubules in each case and also in the collecting tubules in the animals studied three days after injection. Although the transitional type of epithelium contained some lipid material, none was observed in the extremely flat cells.

The concentrations of the nonprotein nitrogen of the blood one, two and three days after a single injection into the portal vein were 34, 55 and 40 mg per hundred cubic centimeters, respectively. The urine of the cats killed one and two days after the injection was essentially normal, that of the cat killed on the third day after the injection contained a trace of albumin, an occasional hyaline and granular cast and from 50 to 60 pus cells per low power field.

EXPERIMENT 4

Observations were made on four dogs killed twenty-four hours after one, two, three and four injections, respectively, into a vein in the leg.

With certain minor exceptions, the changes in the kidneys closely resembled those observed in cats studied under similar conditions. Grossly, there was marked edema. Dilatation of the tubules, although distributed equally widely, was not as marked as in cats receiving the same number of injections. Edema, degeneration and necrosis of the tubular epithelial cells were uniformly present and appeared to be more severe than in the cat. Atypical forms of regenerated epithelium were noted in all cases, but mitotic figures were extremely scarce. A mild inflammatory reaction was present in the interstitial tissue of the medulla and walls of the calices and pelvis, the predominating types of infiltrating cells being plasma cells and mononuclear leukocytes. As in the cat, glomerular changes were inconspicuous.

Small quantities of stainable lipid were present in each case in the cells of the Henle tubules and in three cases (one, two and four injections) in the epithelium of a few convoluted tubules. Fatty casts were present in the medullary tubules in one case (two injections). Stainable lipids were not observed in the atypical forms of regenerated epithelium.

The values for the nonprotein nitrogen of the blood were as follows:

Dog 1—Control, 40 mg, four hours after injection, 49 mg, twenty-four hours after injection, 34 mg.

Dog 2—Control, 28 mg , four hours after injection, 44 mg , twenty-four hours after injection, 52 mg , two days after injection, 38 mg (twenty-four hours after the second injection)

Dog 3—Control, 42 mg , four hours after injection, 56 mg , twenty-four hours after injection, 28 mg , two days after injection, 30 mg (twenty-four hours after the second injection) , three days after injection, 32 mg (twenty-four hours after the third injection)

Dog 4—Control, 37 mg , four hours after injection, 46 mg , twenty-four hours after injection, 33 mg , two days after injection, 29 mg (twenty-four hours after the second injection) , three days after injection, 36 mg (twenty-four hours after the third injection) , four days after injection, 28 mg (twenty-four hours after the fourth injection)

EXPERIMENT 5

Observations were made on twenty-nine cats with total biliary stasis (for from two to eighteen days) killed twenty-four hours after from one to fifteen daily injections into a vein in the leg of 0.2 cc of sodium dehydrocholate (a 20 per cent solution) per kilogram of body weight. The periods of stasis and injection were concurrent, the injections being started twenty-four hours after ligation of the common bile duct.

Gross Observations—After a single injection (stasis of two days) the kidneys were swollen, the cortex was wide and pigmentation was relatively slight. Subsequently the kidneys were frequently of normal size or smaller than normal, the cortex was usually narrow, and pigmentation was rather consistently intense, varying from deep brown to the characteristic grass green seen in total biliary stasis. The surface of the kidney presented a peculiarly mottled appearance, with irregular areas of more intense pigmentation superimposed on a diffusely stained background, the mottling being most conspicuous toward the pelvis, at times these more deeply colored areas tended to become confluent. Edema of the cut surface, although noted occasionally, was in no instance a prominent feature.

Occasionally the green-brown color of the cut surface of the cortex was relieved by pale striations running perpendicularly to the surface of the kidney. In many cases the medulla immediately beneath the cortex presented a narrow red or brownish-red zone of vascular hyperemia, which in some animals extended slightly into the cortex. The tips of the pyramids were usually light or dark brown, the area of medullary tissue intervening between the zone of hyperemia and the tips of the pyramids usually being pale.

Microscopic Observations—Pigmentation. The intensity of pigmentation varied in individual cases, but in general those animals in which biliary stasis was prolonged presented a marked degree of pigmentation more consistently than those in which stasis was of brief duration. In some cases the bile pigment occupied the tubular epithelial cells diffusely, no definite granules being demonstrable, while in others it was present in the form of light yellow or brown granules. The pigmentation was generalized in distribution, all portions of the tubule being involved. Bile-stained casts were noted in various situations, and the desquamated epithelium and precipitated protein material within the lumens were frequently bile-stained.

Dilatation of Tubules. Increase in the outside diameter of the tubules was particularly marked after from six to eight injections but was not a prominent feature during the earlier and later stages of the experiment. Increase in the diameter of the lumen varied considerably, being dependent on the height of the lining epithelium as well as on the outside diameter of the tubule.

Regressive Changes in Tubular Epithelium Marked regressive changes were present in every case. As in the animals without biliary stasis, the intensity and extent of these changes did not increase in proportion to the duration of the experiment but appeared to be related to the relative proportions of typical and atypical forms of epithelium present in each instance. The morphologic characteristics of the degenerated and necrotic cells were similar to those described in connection with these changes in the kidneys of animals without biliary stasis. However, certain minor differences were noted. The homogeneous type of cell was generally more intensely acidophilic, the hydropic, reticulated cells stood out more prominently, and superimposed on these features there was a variable but rather consistently marked degree of bile pigmentation. Bile-stained casts, desquamated, degenerated epithelium, hyaline droplets and albuminous material were frequently seen within the lumens of the tubules. Calcified deposits were rarely observed.

Regeneration of Tubular Epithelium Evidences of regeneration of tubular epithelium were present in every instance, the essential features being identical with those described previously in connection with the groups of animals without biliary stasis. All varieties of the atypical, newly regenerated epithelial cells were observed. The largest proportion of extremely flat cells was noted in an animal receiving seven injections, but relatively large numbers of atypical cells were observed in all cases throughout the course of the experiment. Mitotic figures were exceedingly numerous in all stages after the second injection, in one case as many as thirteen being noted in a single high power field. The regenerating cells were occasionally piled up in syncytial masses.

Interstitial Tissue and Glomeruli The interstitial tissue and glomeruli presented much the same appearance as in the groups of animals described previously. Interstitial fibrosis was never marked, and the glomerular tufts showed no evidence of significant structural damage.

Stainable Lipid The distribution of stainable lipid in twenty-two adult, non-pregnant cats receiving from one to fifteen injections (stasis of from two to eighteen days) was as follows: (1) convoluted tubules only, two cases (a single injection and stasis of two days), (2) convoluted and Henle tubules, ten cases, (3) convoluted, Henle and collecting tubules, seven cases, (4) Henle and collecting tubules, one case, (5) convoluted and collecting tubules, two cases. Stainable lipid was never observed in the extremely flat variety of atypical regenerated epithelium.

Blood Nonprotein Nitrogen—Values for the nonprotein nitrogen of the blood, ranging from 29 to 42 mg per hundred cubic centimeters were obtained in seventeen cats receiving from two to fifteen injections (stasis of from three to eighteen days).

It seems evident that sodium dehydrocholate, injected intravenously, is actively nephrotoxic for the cat and dog. In the cat the renal changes following a single injection into the portal vein were identical, qualitatively, and quantitatively, with those which followed a single injection into a vein in the leg. The process was essentially degenerative, it involved the epithelial cells of the tubules throughout their entire extent and was particularly marked in the region of the corticomedullary junction. There were inconstant and relatively inconspicuous degenerative changes in the glomeruli in some instances and evidences of a mild

inflammatory reaction and fibrosis in the interstitial tissue of the outer medullary zone

Of particular interest were changes in the tubular epithelium indicative of active regeneration. The morphologic characteristics of the regenerated cells were similar to those described by Suzuki,³ Oliver,⁴ MacNider,⁵ Hunter⁶ and MacKay and Oliver⁷ and others in connection with the nephrotoxic effects of a variety of substances, such as uranium nitrate, mercury bichloride, potassium bichromate and sodium phosphate. As early as twenty-four hours after a single injection of sodium dehydrocholate an extensive degenerative lesion was accompanied by the development of a flattened type of newly regenerated epithelium. At this time these atypical cells, although not extremely numerous, were predominantly of the very flat variety. Within a four day period following one injection the intensity of the degenerative process gradually subsided and the number of atypical newly regenerated cells increased, reaching a maximum on the third day, at which time they constituted approximately 35 per cent of the lining cells of the renal tubules. Mitotic figures were also most numerous at this period (three days), being present in association with both mature and atypical forms of epithelial cells. The relative proportion of the extremely flat type of cell steadily diminished, being replaced by cuboidal cells and, on the fourth day, distinctly columnar cells which, although obviously newly regenerated, were approaching normal in size, shape, staining reaction and nuclear characteristics. The impression was obtained that the flat cells, constituting the original regenerative response, gradually and progressively developed into the cuboidal and columnar forms and eventually acquired all the characteristics of mature renal epithelial cells. This is in accord with the observation of Addis and Oliver⁸ of a similar transition from atypical to mature forms of epithelium in animals receiving a single injection of nephrotoxic mineral salts.

The studies of MacNider,⁵ Hunter,⁶ Hunter and Roberts⁹ and others have led to the now generally accepted concept that the atypical form of regenerated epithelium which develops in response to the nephrotoxic action of certain substances (mercury bichloride, uranium salts, potassium bichromate) is resistant to the toxic effects of subsequent admin-

3 Suzuki, T. Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen, Jena, Gustav Fischer, 1912

4 Oliver, J. J. Exper. Med. **21** 425, 1915

5 MacNider, W. deB. J. Exper. Med. **49** 411, 1929

6 Hunter, W. C. Ann. Int. Med. **2** 796, 1929

7 MacKay, E. M., and Oliver, J. J. Exper. Med. **61** 319, 1935

8 Addis, T., and Oliver, J. The Renal Lesion in Bright's Disease, New York, Paul B. Hoeber, Inc., 1931, pp. 125 and 126

9 Hunter, W. C., and Roberts, J. M. Am. J. Path. **9** 133, 1933

istration of these agents. This belief is substantiated by the observations made in the present study. In animals receiving repeated injections of sodium dehydrocholate at intervals of twenty-four hours, regressive changes were not observed in the extremely flat type of immature cell, even though the mature cells throughout the renal parenchyma showed evidences of an advanced stage of degeneration. Immature cells which were apparently transitional between the very flat variety and the mature, columnar form showed degenerative changes to a variable degree but were apparently less susceptible than the original renal epithelium to the nephrotoxic effect of sodium dehydrocholate. After the original exposure, therefore, the morphologic character of the renal response to subsequent injections was influenced to a large extent by the relative proportions then present of three classes of epithelial cells, *i. e.*, (1) those with little if any resistance (original, mature form), (2) those with variable but increased resistance (transition forms of regenerated cells) and (3) those with a high degree of resistance (extremely flat regenerated forms). In view of the fact that after from six to eight injections the great majority of the tubules were lined by the very flat type of epithelium, it is probable that but few of the taller, more mature cells that predominated in the later stages of the experiment were cells originally present in the kidney. It is interesting to note that regressive changes, although present in these taller forms of epithelium after from twelve to fourteen injections, were not as severe or as extensive as those noted after a single injection. This observation is in accord with the hypothesis of diminished susceptibility of the newly regenerated atypical cells to the effects of repeated exposure to a nephrotoxic agent.

The renal changes observed in animals with total biliary stasis were essentially similar to those occurring in animals without obstruction of the common bile duct, with certain exceptions. The severity and extent of degeneration were not significantly influenced by the state of biliary stasis. Regeneration, on the other hand, was apparently more active, mitotic figures particularly being much more numerous. The explanation of this point of difference is not readily apparent for in a previous study^{1c} no evidence of active regeneration and no mitotic figures were observed in the kidneys of cats with total uncomplicated biliary stasis. Bile pigmentation of the kidneys was definitely more intense in animals with biliary stasis receiving dehydrocholate than in those studied previously.^{1c}

The marked dilatation of the tubules, which was a rather constant finding following the administration of sodium dehydrocholate, resembled in certain respects that noted by us^{1c} following decompression of the obstructed biliary system of the cat. However, diminution in the height of the lining epithelium under the latter circumstances was apparently due to actual mechanical stretching rather than to the develop-

ment of atypical forms of regenerated cells. One point of interest and possibly of significance is the fact that calcific deposits, which were not a conspicuous feature in the kidneys of the animals with biliary stasis which received injections, were extremely numerous in one animal with spontaneous decompression. It is possible that calcific deposits in animals with total stasis may have been obscured to some degree by deeply pigmented bile casts.

The effect of sodium dehydrocholate differs from that generally reported in the case of uranium salts in that it is apparently exerted on the epithelium of the renal tubule throughout its entire extent. According to Hunter and Roberts,⁹ regeneration does not usually occur before from four to six days after the administration of uranium and mercury, and Addis and Oliver⁸ stated that the flat, newly regenerated cells begin to assume mature characteristics about the end of the second week. With the administration of sodium dehydrocholate, regeneration apparently occurs much more rapidly, the extremely flat type of cell being present in abundance within twenty-four hours and a gradual transition toward the mature form beginning almost immediately. On the fourth day after a single injection transition forms predominate, comparatively few cells being of the extremely flat variety.

There has been considerable controversy regarding the nature of the mechanism which prevents the markedly atypical forms of regenerated cells from attaining maturity in animals receiving nephrotoxic mineral salts. Addis and Oliver⁸ stated

Although it has been suggested that the cause of this continued abnormality on the part of the developing cells might be sought in some toxic effect of the uranium on the germ plasma of the new cells, to us a more rational and simpler explanation seems to be an interference with their growth or nutrition by the proliferating connective tissue which is at the same time developing side by side with the epithelial structures.

Hunter and Roberts⁹ subscribed to this view. In this connection it is interesting to note that in the present experiments proliferation of connective tissue was practically absent in the cortex, and in this situation there appeared to be little if any inhibition to the progressive development of atypical regenerated cells into mature forms. This observation would appear to support the hypothesis advanced by Oliver.⁴

Modell and Travell¹⁰ found that uranium intoxication in cats was accompanied by the development of an abnormal distribution of stainable lipids in the kidney. These substances, which are normally confined to the epithelium of the convoluted tubules, increased in amount in this situation and, in addition, extended into the cells of the Henle tubules.

10 Modell, W., and Travell, J. *Anat. Rec.* 59: 253, 1934.

A similar abnormality of distribution was reported by us ¹⁴ in cats during biliary stasis and decompression. It was suggested ¹¹ that the appearance of lipids in the epithelium of the Henle and collecting tubules probably was not a part of a regressive process but rather was related to metabolic changes or to an alteration in the functional activity of the tubular epithelium. The observations in the present experiment resemble those previously described in animals with nephritis due to uranium and with biliary stasis and decompression. Because of the presence of degenerative changes throughout all portions of the renal tubule, no further light is thrown on the pathogenesis of the altered distribution of lipid. It is of interest to note that stainable lipid was rarely observed in the extremely flat variety of the atypical regenerated cell, increasing in amount as the cell became more mature. This may be related in some way to the development of functional activity by these cells.

In many instances the presence of functional or morphologic renal injury was evidenced by the appearance of albumin and casts in the urine. The fact that the most marked grades of nitrogen retention were generally observed within twenty-four hours after the first injection of sodium dehydrocholate suggests that the most severe functional injury is associated with the extensive initial degenerative changes in the original renal epithelial cells. The subsequent subsidence of the level of the nonprotein nitrogen of the blood in most cases, despite repeated injections of dehydrocholate, suggests that the atypical regenerated cells possess some degree of functional activity. Unfortunately, no functional studies were made after a single injection in animals with biliary stasis, however, the absence of nitrogen retention in seventeen such animals receiving from two to fifteen injections was in striking contrast to its presence in four of ten cats without biliary stasis which received multiple injections. It is of interest in this connection that the activity of regeneration was generally greater in the former than in the latter.

In view of the numerous observations of the pronounced choleretic effect of sodium dehydrocholate and in view of the probability that it is eliminated, to some extent at least, in the bile it seems strange that relatively little difference in nephrotoxic effect was noted in the animals with and without biliary stasis. The question may be raised whether this substance may not undergo repeated excretion and reabsorption by the kidney, its elimination by the liver occurring gradually. This hypothesis is strengthened by the observation that the changes in the kidney which followed the injection of dehydrocholate into the portal vein were practically identical with those which followed its injection into a peripheral vein.

The clinical literature contains many references to a diuretic effect of sodium dehydrocholate in patients with edema associated with con-

11 Stewart, Cantarow and Morgan ¹⁰ Modell and Travell ¹⁰

gestive heart failure and other conditions accompanied by hepatic functional impairment. This diuresis has been explained on theoretical and speculative grounds, usually being attributed to improvement in hepatic function, with consequent restoration of water balance. Despite sporadic reference to the observation of swelling of the kidneys in experimental animals we were unable to find any reference to a detailed morphologic study of the kidneys following the administration of this substance. It seems to us that the clinically and experimentally observed diuresis may be explained more rationally on the basis of the development of a renal tubular lesion, being similar in this respect to the diuresis which commonly occurs soon after the administration of uranium nitrate.

SUMMARY

The intravenous administration of sodium dehydrocholate in the cat and dog results in a severe renal lesion characterized by regressive and regenerative changes in the tubular epithelium. Changes following injection into the portal vein were similar to those which followed injection into a peripheral vein. Advanced grades of nitrogen retention were observed twenty-four hours after a single injection.

The process of regeneration was characterized by the development of atypical forms of renal epithelium which were more resistant than the original epithelium to the toxic effects of sodium dehydrocholate and which appeared to develop into mature forms morphologically indistinguishable from the original tubular cells.

The characteristic renal lesion was essentially the same in animals with and without biliary stasis which received injections although mitotic figures were much more numerous in the former.

There was an alteration in the distribution of stainable lipid, which extended into the cells of the Henle and the collecting tubules, similar to that described in cats with nephritis due to uranium and with biliary stasis.

These observations suggest that the clinically observed diuretic effect of sodium dehydrocholate may be dependent on the development of a renal tubular lesion and may be comparable to the diuresis that occurs soon after the administration of uranium salts. Although the renal changes may possibly represent a peculiarity of species, their consistent occurrence in both the cat and the dog following the administration of sodium dehydrocholate in ordinary therapeutic dosage renders the advisability of the clinical employment of this substance doubtful, at least until more evidence is at hand of its innocuousness for man.

Case Reports

DUPLICATION OF THE SPINAL CORD, WITH SPINA BIFIDA AND SYRINGOMYELIA

ARTHUR WEIL, M.D., AND WARREN B. MATTHEWS, M.D., CHICAGO

Malformations of the spinal cord are of interest not only to the pathologist but to the embryologist, who from such arrests and aberrations of development may draw important conclusions as to the early stages of growth of the central nervous system. The present case is one of the rare instances of duplication of the lower segments of the spinal cord, and the anomaly does not belong to the large group of artefacts in which Van Gieson, somewhat radically, wanted to place most such malformations which had been reported prior to his time¹. The case may be classified with the relatively few instances of duplication of the spinal cord which has its origin in a discrepancy of embryonic growth produced either, as the older theories explained, by lack of closure of the open neural tube and folding of its lateral walls or, as newer investigations suggest, by lack of fusion of the two separated tubes of the solid anlage of the lumbar segments into one central canal.

REPORT OF A CASE

History—The spinal cord of a child, 5 months old, was obtained through the cooperation of Dr. Philip Kreuscher. Operation had been performed for the relief of spina bifida, and an attempt had been made to remove the meningocele. But an ascending infection of the urinary tract developed and pyelonephritis and death followed.

Anatomic and Histologic Report—Grossly, the cervical segments of the spinal cord showed no abnormalities. At the middle of the thoracic region the spinal cord bulged. The posterior wall of the cord at this level was ruptured, and the opening led into a cavity which extended through approximately three segments. At the lower end of the dorsal segments the spinal cord was divided into two separate cords, which at the cauda equina were surrounded by thick scar tissue consisting of muscles and connective tissue (fig. 1).

Cervical Enlargement—Sections from this level showed a well developed spinal cord, which, however, was exceedingly flat. The anterior horns differed

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¹ Van Gieson, I. New York M. J. 56: 336, 1892.

in size. The smaller contained fewer, more elongated and smaller neurons than the horn of the other side. The central canal was widened and lined with an inner row of large cylindric cells with cilia on the inner surface and an outer row of slender cells with long processes extending into the spinal cord (fig 2). This type of lining of the central canal with similar primitive ependymal cells and supporting spongioblasts was observed throughout the whole spinal cord and in the halves of the duplication. Both the anterior and the posterior roots were present and contained myelinated fibers.

Upper Thoracic Segments Sections from this level showed a microscopic picture similar to that already described. In the posterolateral column at one side there was an area of glial proliferation consisting in the peripheral portion of a loose meshwork of fibers and numerous glia cells, among them were adult astrocytes intermingled with more primitive, spongioblast-like forms and large cells without processes, the nuclei of which contained different stages of mitotic division (fig 3). The center of this area was necrotic, and in some sections a central cavity (syrinx) had developed.

Midthoracic Segments Sections from this level revealed a marked hydromyelia, which had compressed the spinal cord to such an extent that only a thin

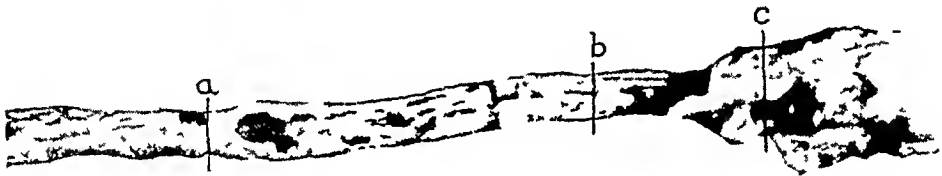


Fig 1—Posterior surface of the spinal cord. The transverse lines *a*, *b* and *c* indicate the approximate level from which the microscopic sections were taken, photographs of which appear in figures 4, 5 and 6, respectively.

wall of nerve tissue remained (fig 4). In other sections the posterior wall of the cavity was ruptured (fig 1). More caudal sections showed gradual division of the central canal into two portions and gradual separation of the spinal cord into three parts, set off by pia-arachnoid. The middle part showed no specific structure but contained only glia, while the lateral and larger parts possessed the structure of a small spinal cord, as described later. At this level the posterior wall of the central canal, which, like the canal in the other segments with hydromyelia, had been deprived of its cellular lining, showed a wartlike protrusion of proliferated glia tissue.

Lower Dorsal Segments Sections from this level revealed that the spinal cord had been divided into halves, which formed mirror images and were turned at an angle of 90 degrees with their anterior fissures facing each other. In each small spinal cord a widened central canal was present, lined with primitive ependymal cells. In each cord the two anterior horns were separated, but the posterior horns had been united into one, from which a posterior root originated at about the middle of the lateral portion of the circumference (fig 5). Only one anterior horn, the one situated more anteriorly, was well developed, it contained normal

neurons and sent out an anterior root. The other anterior horn was considerably smaller, it contained fewer and smaller neurons and did not send out axons toward the periphery. Both the anterior and the posterior commissure were well developed. Only the column of Clarke next the well developed anterior horn had normal ganglion cells. Occasionally, isolated ganglion cells could also be seen in the region of the opposite column of Clarke. The central canal of both halves was widened and lined with embryonic ependymal spongioblasts. The outer zone of marginal glia (Held) was rather wide and contained many large astrocytes and fewer oligodendroglia and microglia cells. Each spinal cord had a well

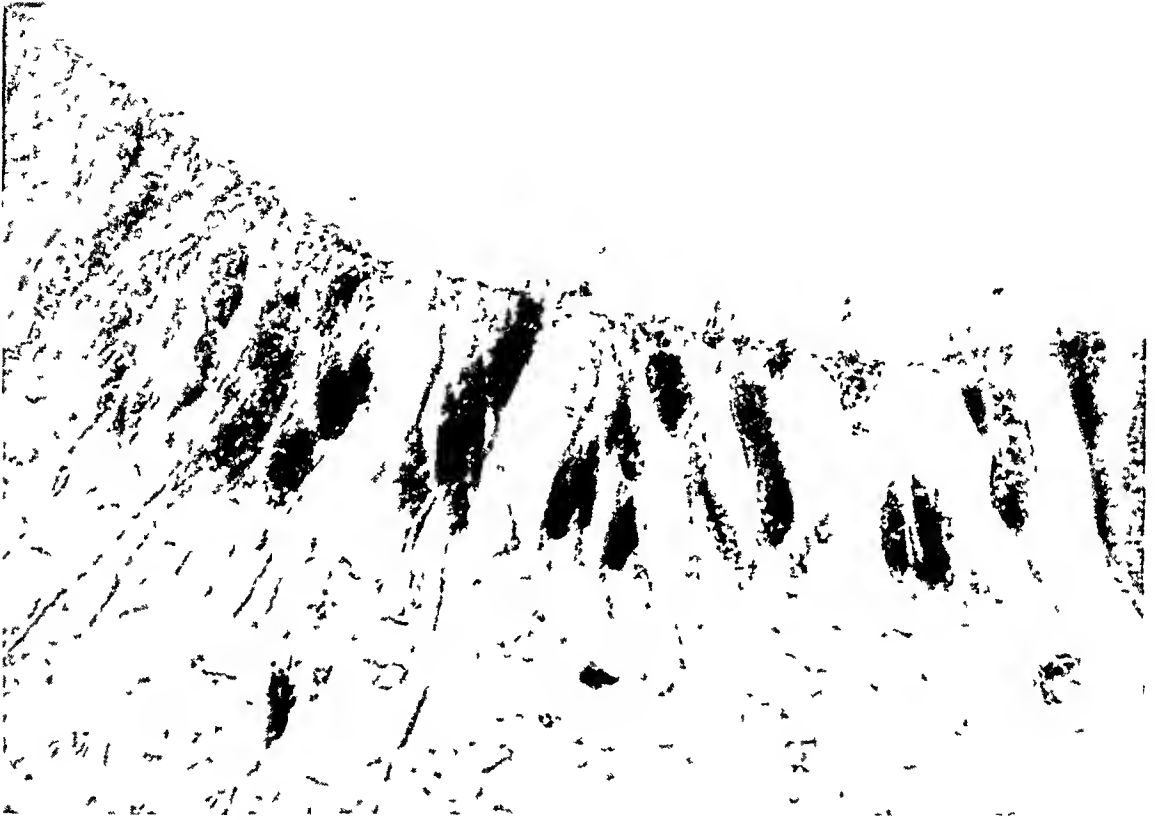


Fig 2—Photomicrograph of the lining of the central canal of an upper dorsal segment. Ciliated cells and an outer row of supportive spongioblasts which send out long processes toward the periphery are demonstrated. Davenport stain. Zeiss objective 3 mm, ocular 10 X.

developed anterior fissure (pointing toward the midline) with separate blood vessels and pia-arachnoid. Furthermore, the two spinal cords were surrounded by a common sheath of connective tissue, with a large anterior spinal artery and vein.

Sacral Segments. Sections from this level revealed that the halves of the spinal cord were completely separated by dense masses of connective tissue, which also surrounded well myelinated nerve roots. In neither half was there a definite pattern of arrangement of the gray and white matter. The central canal was wide and lined as already described. Numerous small blood vessels were present,



Fig 3—Photomicrograph of a section from an upper dorsal segment, showing (A) formation of a glioblastoma with beginning central necrosis (syringomyelia) (Van Gieson stain, Zeiss objective 10 \times , ocular 10 \times and (B) mitotic figures in neoblastic glia cells in the same section (Zeiss objective 3 mm, ocular 10 \times)



Fig 4—Transverse section through a midthoracic segment, showing hydromyelia with destruction of the lining of the posterior wall of the central canal Van Gieson stain, Zeiss objective 3 \times , ocular 10 \times , reduced approximately 85 per cent

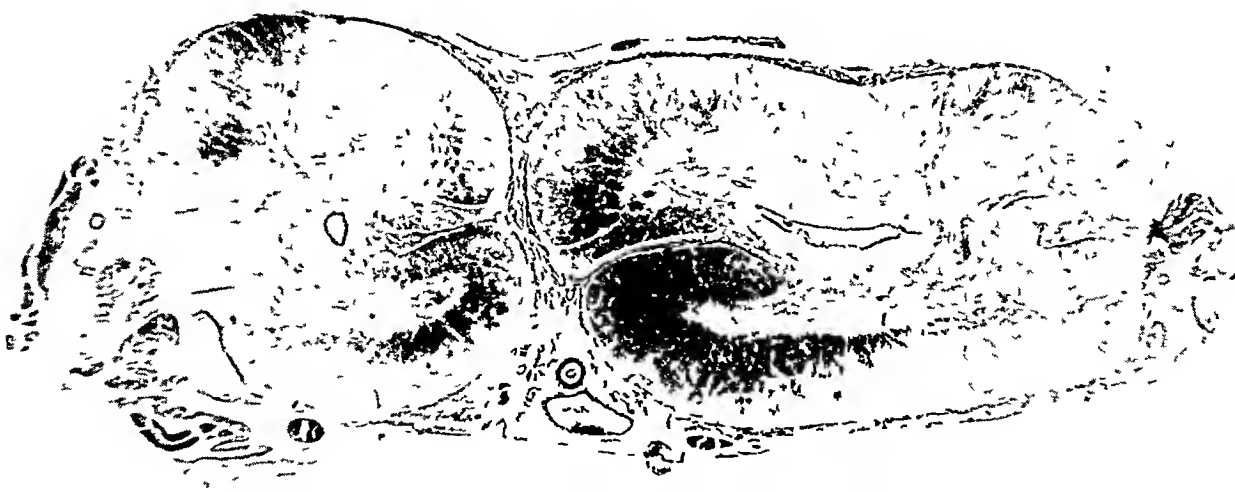


Fig 5—Transverse section through a lower dorsal segment, showing duplication of the spinal cord To the right a posterior root is seen emerging from the posterior horn Davenport stain, Zeiss objective 15 \times , ocular 6 \times

surrounded by areas of proliferated glia, which in places showed beginning necrosis with the formation of a syrinx (fig 6)

COMMENT

A review of the literature on duplication of the spinal cord published up to 1926 has been given in a previous paper² Since then few cases of this malformation have been reported Altschul³ described such a duplication of the spinal cord in the upper dorsal segments of a child

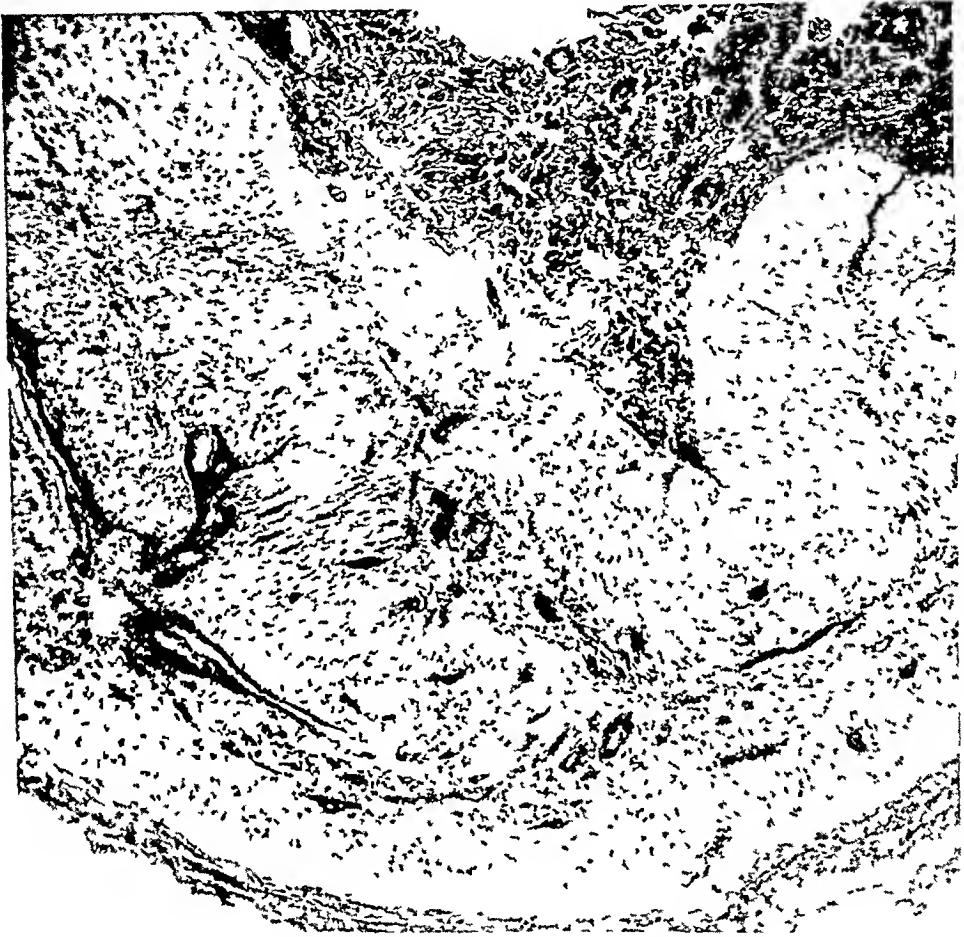


Fig 6—Transverse section through a sacral segment, showing marked increase in the number of blood vessels and thickening of the pia-arachnoid Van Gieson stain, Zeiss objective 3 X, ocular 6 X

9 months old Each half contained a separate central canal, which was gradually united to the canal of the other half at the level of the twelfth dorsal segment, below which a normal spinal cord was again present Unfortunately, no detailed description of the lining of the central canal was given but it appears from the photomicrographs taken with low

² Kraus, W M, and Weil, A *Rev neurol* 2 317 (Oct) 1926

³ Altschul, R *Virchows Arch f path Anat* 271 45, 1929

magnification that in this case, too, large cylindric cells were present. It seems that Altschul misinterpreted the French text of the publication of Kiaus and Weil.² He cited them as "being inclined, on the basis of their own observation of an artificial duplication, to think that all malformations of the spinal cord are artefacts, according to the theory of Van Gieson." In reality, Kiaus and Weil stated "No case of duplication of the spinal cord as a whole has been reported in the literature which can be traced to a double embryonic anlage. The duplications described had their origin in the formation of embryonic fissures, foldings of the medullary plate, separation of the spinal cord by new formations of the vertebral column and ingrowth of connective tissue or of tumors (dermoids)."

A second case of spina bifida combined with acrania and rachischisis was reported by Schenk.⁴ His description corresponds exactly to that in the present case. The two separated spinal cords had been turned toward the midline at an angle of 90 degrees and represented mirror images. Each had the structure of a normal spinal cord with well developed horns of gray matter and a central canal. In a third publication von Santha⁵ described a duplication confined to the lumbar segments. Both spinal cords had the normal position of the cord in the adult and the second, smaller, division was superimposed on the posterior part of the larger. If each spinal cord had not contained a separate central canal one might have thought judging from the drawings of sections of the lumbar segments, that perhaps one was dealing with an artefact—a pseudoheterotopia.

The present case is of interest on account of the manifold evidences of retardation of development. Unusual is the preservation of ciliated ependymal cells and supportive spongioblasts in a child 5 months old. Furthermore the presence of mitotic figures in the nuclei of the foci of proliferated glia indicates that the embryonic tendency to growth had not yet been arrested or led into adult channels. The position of this center of growth close to the central canal is in accordance with the normal embryonic growth of the spinal cord which starts from germinal centers close to the central canal and from which newly formed spongioblasts and neuroblasts migrate toward the periphery. The formation of a central cavity of necrosis within the foci of glial proliferation in the upper dorsal segments sheds an interesting light on the development of syringomyelia. It supports the prevalent theory that syringomyelia arises from the central necrosis of an intramedullary glioblastoma. This neoplasm may develop from embryonic rests of spongioblasts which remain close to the midline.

4 Schenk, V. W. D. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **146**: 369, 1933.

5 von Santha, K. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **123**: 753, 1930.

and posterior to the central canal in the upper dorsal and the lower cervical segments. The presence of mitotic figures and the active proliferation of glia cells with the formation of primitive spongioblasts indicate that in this case a real neoplastic formation was present, a glioblastoma and not merely a reactive gliosis stimulated by the pressure atrophy of the spinal cord following the marked hydromyelia. The degree of hydromyelia must have been considerable in the segments below these foci of syringomyelia, because it produced such marked thinning of the substance of the spinal cord that the thin, bulging wall ruptured during careful removal of the cord. The marked overgrowth of blood vessels in the sacral segments and the surrounding gliosis may have been the first step toward the formation of a hemangioma, which occasionally is noted in this region. Besides, there was a tendency toward abnormal growth of the pia-arachnoid. Considerable thickening occurred throughout this membrane, which assumed massive proportions in the lower part of the sacral region and around the cauda equina.

Another interesting feature in this case is the development of the anterior and posterior horns of gray matter and their corresponding nerve roots in the duplicated segments. Though there were two anterior horns in each half, only the more anteriorly situated contained well developed neurons and gave rise to an anterior root. From the second, underdeveloped, anterior horn of gray matter no axons were seen streaming peripherally. In some sections there was the suggestion of two posterior horns in each half, but the one situated more posteriorly usually was atrophic. In most sections the two horns were combined, and only one posterior root arose from it, near the middle of the lateral surface of the cord. These observations are of considerable interest to the embryologist. They testify to the development of spinal nerve roots and muscle metameres independent of the development of the spinal cord. In this connection may be cited the case reported by Sokolansky,⁶ who described the absence of the spinal cord in a fetus in the presence of well formed posterior spinal nerve roots and ganglions. In the present case, furthermore, only one well formed column of Clarke was present in each half of the duplicated cord. It was situated on the side of the exit of the posterior nerve root, i. e., in the anterior half of each duplication. The intersegmental nerve connections of each half of the spinal cord were well developed. There were an anterior and a posterior commissure containing myelinated fibers, and the intersegmental fiber tracts were well formed. With the exception of a rather wide zone of marginal glia there was no abnormality in the formation of the posterior, lateral and anterior columns which contained numerous myelinated fibers.

⁶ Sokolansky, G. *Arch f Psychiat* 92:354, 1930

Many theories have been advanced to explain such a duplication of the spinal cord associated with rachischisis. Riley has discussed all the theories in detail.⁷ Most former investigators assumed that the whole spinal cord arises from an open neural tube, and they concluded, therefore, that failure of the tube to close and subsequent folding of the isolated lateral walls of the tube resulted in such a duplication. More recently, Holmdahl,⁸ Ikeda⁹ and others suggested that early in embryonic life the caudal part of the spinal cord develops not from an open tube but from a solid bundle of cells in which two or more cavities are formed. Later, the two tubes become confluent, and the united cavities form the central canal. Failure of such a fusion results in persistent duplication of the lower segments of the spinal cord, around each cavity nerve tissue forms a spinal cord, which is separated from its duplicate by ingrowing connective tissue. In the midthoracic or the upper dorsal region the two separated central canals gradually become confluent as was illustrated in Altschul's case and also in ours.

SUMMARY AND CONCLUSIONS

A case of duplication of the spinal cord beginning in the lower dorsal segments associated with spina bifida is described. Besides, marked hydromyelia of the midthoracic segments, a central canal lined by ciliated ependymal cells and supportive spongioblasts and formation of a glioblastoma, with central necrosis and syringomyelia in the upper dorsal and the lower cervical segments, were present.

7 Riley, H. A. *J. Nerv. & Ment. Dis.* **72** 1, 1930.

8 Holmdahl, D. E. *Morphol. Jahrb.* **54** 333, 1925; **55** 112, 1925.

9 Ikeda, Y. *Ztschr. f. Anat. u. Entwicklungsgesch.* **92** 380, 1930.

ARSENIC NECROSIS OF THE STOMACH AFTER INTRAVENOUS INJECTION OF NEOARSPHENAMINE

OSCAR O CHRISTIANSON, M D, CHICAGO

Among the more serious lesions occasionally produced by the intravenous administration of arsenic are profound changes of the hematopoietic tissues of the bone marrow, such as aplastic anemia, agranulocytosis and purpura haemorrhagica, hemorrhages of the brain, termed hemorrhagic encephalitis, and acute retrogressive and fatty changes of the liver. Hemorrhages, according to Petri,¹ are the commonest lesions observed post mortem in these cases. They occur in the brain, heart, pleurae, serosa, lungs, kidneys, mouth and lining of the gastro-intestinal tract.

A review² of the reports on 72 syphilitic patients whose deaths resulted from complications due to the intravenous administration of compounds of organic arsenic, yields the following conclusions. Fourteen died of aplastic anemia, 7 of agranulocytosis, 4 of purpura haemorrhagica, 4 of acute yellow atrophy of the liver, 2 of necrosis and hemorrhages of the kidneys, eighteen of hemorrhagic encephalitis and 25 of complications apparently due to arsenic therapy, but without characteristic changes in the viscera. In 38 cases there were no appreciable changes in the lining of the stomach or intestinal tract, in 23, petechial hemorrhages of the gastro-intestinal tract, in 8, similar changes of the stomach alone, and in 2, hemorrhages only of the lining of the small bowel. Herzog³ published the only report of a case in which the stomach showed marked changes, consisting of extensive erosions, hemorrhages and edema of the lining and in which ulcerations were present in the large bowel. His patient had received eleven intravenous injections of neoarsphenamine, the last about one month before death.

According to these statements, edema and petechial hemorrhages of the lining of the stomach are the usual changes noted in cases of arsenic poisoning due to intravenous medication. The extensive necrosis associated with edema and hemorrhages, described in the following report is rare.

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1 Petri, E., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol. 10, p. 159.

2 These statements were compiled from forty-five references collected from the *Quarterly Cumulative Index* and the *Quarterly Cumulative Index Medicus* for the years 1917 to 1933, inclusive.

3 Herzog, G. *Munchen med. Wchnschr.* 65:29, 1918.

REPORT OF CASE

A white man aged 46 entered St Luke's Hospital on July 18, 1934, because of bleeding from the gums for three days and general weakness. He had petechiae of the palate and buccal and pharyngeal mucosa and a purpuric eruption of the skin of the thighs and legs. The temperature was 100.2 F. The blood count showed 3,310,000 erythrocytes, 750 leukocytes and 3,300 platelets per cubic millimeter. The differential examination revealed 2 per cent band-shaped polymorphonuclears, 6 per cent eosinophils, 2 per cent monocytes, 90 per cent small and large lymphocytes and no mature polymorphonuclears. The bleeding time and coagulation time were fifteen and four minutes, respectively. The hemoglobin content was 58 per cent (Sahl). The urine was unchanged. The clinical diagnosis was agranulocytosis. Three blood transfusions and daily intramuscular injections of pentnucleotide and liver extract during ten days made no appreciable change. Death occurred on July 28.

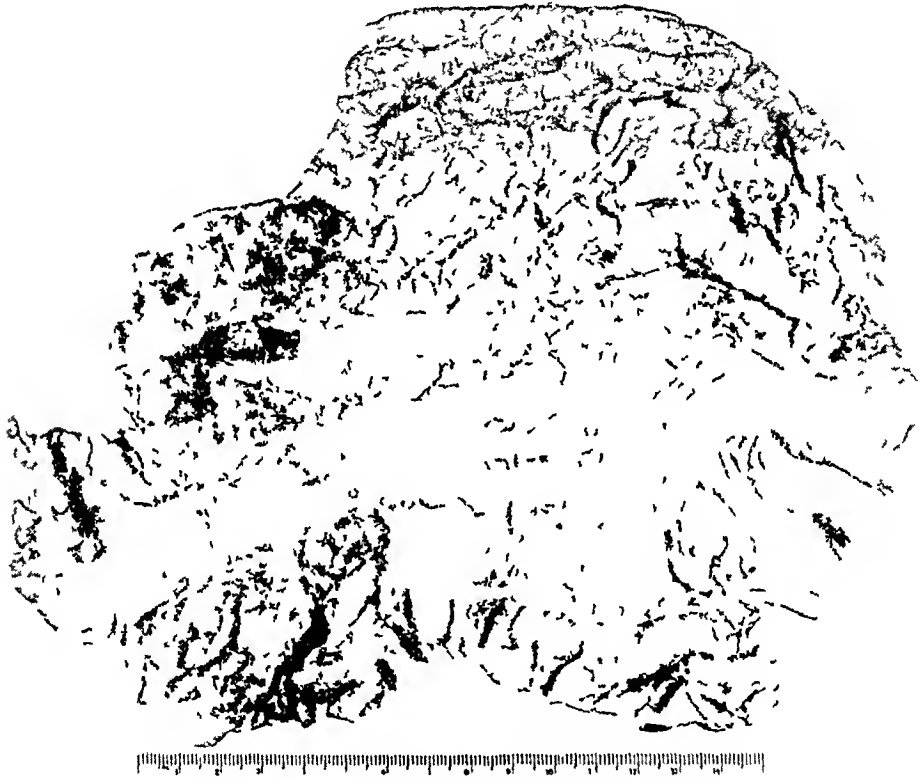
The patient had visited a public clinic in February because of weakness and mental changes. The serologic tests for syphilis were positive, and tests of the spinal fluid were negative. In 1922 a Wassermann test of the blood made at the same clinic was negative. During February and March 1934 the patient was given five intramuscular injections, each of 0.2 Gm, of bismuth subsalicylate at intervals of one week, and four intravenous injections, each of 3 Gm, of sodium iodide. For the next six weeks he was given weekly intravenous injections of neoarsphenamine, the average dose being 0.6 Gm, and weekly intramuscular injections of 0.2 Gm of bismuth subsalicylate. From May 9 to June 6 the patient was not treated, and the Wassermann reaction of the blood was negative. On June 6 treatment was resumed, with four intramuscular injections of bismuth subsalicylate at intervals of one week and three intravenous injections of neoarsphenamine in the dosage mentioned. In all, 5.4 Gm of neoarsphenamine was injected in approximately three months. He had no reaction until three days before entering the hospital—nineteen days after the last injection of neoarsphenamine.

The gross and microscopic postmortem examination revealed marked chemical (arsenic) pseudomembranous necrosis and hemorrhages of the lining of the stomach, signs of recent hemorrhage into the lumen of the stomach and the small and large bowel, acute ulcerative gingivitis, petechial hemorrhagic areas in the mucous membranes of the soft palate, pharynx, epicardium, lining of the inferior vena cava and lining of the large bowel, ecchymoses of the skin of the arms and legs, slight edema of the brain, central necrosis of the lobules and fatty changes of the liver, aplasia of the bone marrow, bronchopneumonia of the right lung, cloudy swelling of the myocardium, liver and kidneys, and marked anemia.

The lining of the stomach had large, markedly edematous rugae extending parallel to the long axis, varying from 0.5 to 2 cm in width and projecting into the lumen about 1 cm (fig). These rugae and small, scattered, discrete portions of the lining of the stomach were covered by a gray-brown, firmly adherent pseudomembrane with many small and large red-brown hemorrhagic erosions and petechial hemorrhages. The mucosa beneath the membrane was necrotic. The gastric mucosa that was not covered by a membrane was dull, gray-red and edematous. The surfaces made by cutting the large rugae transversely had necrotic edematous mucosa, and the submucosa formed a wide irregular layer of red-brown tissue with many large hemorrhages. The edema of the tissues and the marked hemorrhages into the submucosa, although present in all portions of the stomach, were most marked in the rugae. There were no changes in the esophagus or the

duodenum An eroded red-gray ridge on the lesser curvature, 2 cm proximal to the pyloric ring, measured 5.5 by 3.5 by 1.5 cm In the submucosa beneath was a large contracted blood clot The serosa of the stomach seemed smooth

Histologically, the mucosa and especially the submucosa of the stomach were markedly edematous The surface of the lining was necrotic and covered by a pseudomembrane that was in places 1.5 mm thick It consisted of necrotic mucosa with slightly discernible structural details and a large amount of fibrin enmeshing desquamated cells, masses of red blood cells and brown blood pigment The markedly edematous submucosa had extensive extravasations of red blood cells, dilated blood capillaries and deposits of hemosiderin The walls of many small arterioles stained poorly, and the endothelial cells of many were swollen and



Corrosion, pseudomembranous necrosis and hemorrhages of the gastric mucosa

loosened from the wall of the vessel There were small collections of lymphocytes about a few arterioles in the mucosa and submucosa The muscle and serosal layers had no noteworthy changes

The liver and kidneys were analyzed by Dr C W Muehlberger, the coroner's chemist The liver contained 0.666 Gm of arsenic, and the kidneys, 0.1255 Gm The patient had received 5.4 Gm of neoarsphenamine, or an equivalent of 1.946 Gm of arsenic Accordingly, the amount of arsenic recovered from these viscera was 40.36 per cent of the amount administered intravenously, presuming the arsenic medication was reported correctly Considered in terms of unit weight, the liver had a slightly larger amount of arsenic than the kidneys No mercury or bismuth was found in the viscera examined Stained preparations of the necrotic mucosa showed a mixture of staphylococci, yeast cells and fusiform bacilli

COMMENT

Various theories have been offered to explain the occasional corrosive-like changes erosions, hemorrhages and edema of the mucosa of the gastro-intestinal tract following the oral or intravenous administration of various compounds of arsenic. Because of these changes in the mucosa, arsenic has been regarded as a corrosive poison. The usual changes of the lining of the gastro-intestinal tract of patients who have died from the effects of acute or chronic arsenic poisoning however, are hyperemia, petechial hemorrhages and edema. Schaumburg, according to Merkel,⁴ in 1893 reported such changes in 150 of 197 cases of acute arsenic poisoning. Pistorius⁵ administered large doses of arsenous acid intravenously and orally to cats and dogs without producing corrosion of the lining of the gastro-intestinal tract. Unterberger and Boehm,⁶ in similar experiments, produced pseudomembranes of the lining of the small intestine but not of the stomach.

The excretion of arsenic has been thought to be mainly by the mucosa of the gastro-intestinal tract. The hyperemia, edema, petechial hemorrhages and other changes following intravenous medication were explained according to that view. Lesser⁷ in 1878 ligated the esophagus of a dog, then injected arsenous acid intravenously and subsequently noted arsenic in the gastric and intestinal contents. He concluded that arsenic is excreted by the lining of the gastro-intestinal tract.

Others have demonstrated that arsenic is excreted into the bile in large quantities. Kolls and Youman's⁸ injected neoarsphenamine intravenously into twenty dogs. In three of these the common bile duct was ligated, and the bile was collected. It was found that approximately eight times as much arsenic was excreted in the bile as in the urine. Arsenic was noted in the stomach content only when bile was present. Bulmer⁹ obtained the same results. He noted traces of arsenic in the intestinal contents of dogs with ligated bile ducts, but he considered that it was due to contamination. Within five minutes after injection, the liver contained the largest amount of arsenic, but later and for several days it contained less than the lungs presumably because of the large amount excreted into the bile. According to Sollmann¹⁰ also most of

4 Merkel, Hermann, in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 4, p. 294.

5 Pistorius, Hugo. *Arch f exper Path u Pharmacol* **16** 188, 1882.

6 Unterberger and Boehm, R. *Arch f exper Path u Pharmacol* **2** 89, 1874.

7 Lesser, A. *Virchows Arch f path Anat* **74** 133, 1878.

8 Kolls, A. C., and Youmans, J. B. *Bull Johns Hopkins Hosp* **34** 181, 1923.

9 Bulmer, J. M. R. *J Pharmacol & Exper Therap* **21** 301, 1923.

10 Sollmann, T. *A Manual of Pharmacology*, Philadelphia, W. B. Saunders Company, 1926 p. 965.

the arsenic is excreted through the bile, the first 60 per cent rapidly and the remainder slowly

Arsenic, therefore, does not act directly on the lining of the stomach or intestinal tract as a corrosive or irritant, but in toxic doses it produces a vasoparalysis and vasodilatation of the small arterioles and capillaries of the mucosa and submucosa. This vasoparalysis results from its toxic action on the innervation of the capillaries. Boehm, Unterberger and Pistorius were among the first to note the vasoparalytic action and subsequent marked drop in blood pressure when toxic doses of arsenous acid were administered orally or intravenously to dogs. The vasoparalysis caused marked circulatory stasis and sometimes produced thrombi. Following the vasodilatation, hemorrhages and marked exudation of plasma were noted. These changes, they stated, seriously impede the blood supply of the overlying mucosa. The mucosa becomes necrotic and together with the exudates and fibrin forms the pseudomembrane. The mucosa thus seems excoriated or corroded.

The patient whose case has been reported probably was sensitive to neoarsphenamine. The gross and microscopic changes noted were compatible with lesions caused by a primary vasoparalysis of the small blood vessels in the gastric mucosa and submucosa. There appears to have been delayed excretion of arsenic, because, according to chemical analyses, 40.36 per cent of the arsenic was recovered in the kidneys and liver thirty-one days after the last injection of neoarsphenamine.

The micro-organisms that were present in the necrotic gastric mucosa are believed to have been secondary invaders, similar to the flora commonly found in the saliva of the mouth.

SUMMARY

This report describes marked arsenic necrosis and hemorrhages of the lining of the stomach of a patient who had been treated for syphilis with intravenous injections of neoarsphenamine in moderate doses. Thirty-one days had elapsed since the last injection of neoarsphenamine.

Laboratory Methods and Technical Notes

THE APPLICATION OF THE GRAM STAIN TO PARAFFIN SECTIONS

JOHN H. GLYNN, M.D., MONTREAL, CANADA

Although Christian Gram¹ originally devised his staining method for the demonstration of bacteria in tissues, it is doubtful if he recognized the existence of bacteria which were decolorized by this method. The use of a counterstain was a later introduction which led to the concept of gram-positive and gram-negative organisms (Conn²). At the present time the Gram stain is important to bacteriologists because it immediately provides a broad classification of bacteria and shows their morphology as well. Its usefulness, however, has been largely limited to smears. When the stain is applied to tissue sections the results are disappointing.

Weigert's³ method for the staining of fibrin and gram-positive bacteria gives good histologic results and is still widely in use, but it does not stain gram-negative organisms. MacCallum⁴ recommended a combination of Weigert's and Goodpasture's stains, which differentiate gram-positive and gram-negative bacteria. Other methods accomplishing the same purpose have been reported by Lillie,⁵ Scudder and Lisa,⁶ Brown and Brenn⁷ and Rudnikoff and Stawsky.⁸

The use of trinitrophenol in some of these methods suggests that the failure of the usual Gram stain to give satisfactory histologic results may lie in the unsuitable concentration of hydrogen ions at which the stains are applied. Experiment justifies this assumption.

A series of paraffin sections of the same material containing both gram-positive and gram-negative bacteria was stained as follows:

Each section was brought through xylene and alcohol to water in the usual way. Gentian violet was applied for one minute, followed by compound solution of iodine for one minute, and the sections were decolorized with 95 per cent alcohol and then washed in water. Buffered solutions at pH varying from 1 to 13 were applied for one minute. Dilute carbol-fuchsin was added for one minute to the buffered solutions on the slide. The sections were differentiated and dehydrated rapidly in acetone, cleared in xylene and mounted in balsam.

From the Department of Bacteriology, McGill University

1 Gram, C. *Fortschr. d. Med.* **2**: 185, 1884.

2 Conn, H. J. *Stain Technol.* **5**: 46, 1930.

3 Weigert, C. *Ztschr. f. wissenschaftl. Mikr.* **4**: 512, 1887.

4 MacCallum, W. G. *J. A. M. A.* **72**: 193, 1919.

5 Lillie, R. D. *Arch. Path.* **5**: 828, 1928.

6 Scudder, S. A., and Lisa, J. R. *Stain Technol.* **6**: 51, 1931.

7 Brown, J. H., and Brenn, L. *Bull. Johns Hopkins Hosp.* **48**: 69, 1931.

8 Rudnikoff, I., and Stawsky, H. *Arch. Path.* **19**: 543, 1935.

At p_H 7 and above, the tissue as a whole was a diffuse red, the histologic character being difficult to make out, and no differentiation between gram-positive and gram-negative organisms being shown. Below p_H 7, the differentiation of bacteria and histologic structure became increasingly clear with increasing acidity until at a p_H between 2 and 3 the most satisfactory preparations were obtained. At p_H 2 and below, basic fuchsin itself becomes decolorized. Similarly, at p_H 2 and below, gram-positive organisms (except the acid-fast variety) lose the gentian violet, as can be demonstrated by washing in water and subsequently staining with basic fuchsin at p_H 3 or 4.

The experiment demonstrates that the Gram stain can be applied successfully to tissue sections if the p_H at which the basic fuchsin is employed is controlled. Buffered solutions are unnecessary. The commonly used Gram stain for smears can be applied successfully to sections if the section is treated with hundredth-normal hydrochloric acid for one minute before the application of the basic fuchsin.

As far as the staining reactions of the bacteria are concerned, this result might have been predicted from the work of Stearn and Stearn,⁹ who found an iso-electric staining range for gram-positive bacteria in the neighborhood of p_H 2 to 3 and a somewhat wider range centering about p_H 5 for gram-negative bacteria.

The problem of securing satisfactory histologic detail simultaneously with good bacterial differentiation introduces the question of decolorizer. Churchman's¹⁰ statement that the "ability to retain the dye when stained by the method of Gram is not a property of living cells in general but is almost entirely confined to yeasts and bacteria" must be regarded as more relatively than absolutely true. When alcohol is used as decolorizer, more or less of the violet is retained by the cell nuclei. As Lillie⁶ pointed out, acetone is a more effective and more rapid decolorizer for tissue sections. Moreover, he compared the effects of alcohol and of acetone as decolorizers on smears of eighty-three different organisms. When discrepancies occurred, the results after the use of acetone were more often nearer to the accepted Gram reaction for the organism in question.

Lillie's contention as to the effectiveness of acetone as a nuclear decolorizer has been verified in the present study. The importance of the iodine might be mentioned, for, if it is omitted, even acetone fails to remove the violet from the nuclei. If iodine forms a precipitate with the gentian violet which is adsorbed on nuclei as well as on bacterial surfaces, this precipitate is removed by acetone from nuclei and gram-negative bacteria within ten seconds, but not from gram-positive bacteria. Evidence that this behavior may be related to lipid substances is found in the observation that the myelin sheaths of nerve cells retain much of the violet after being washed with acetone following staining with gentian violet and iodine.

⁹ Stearn, A. E., and Stearn, E. W. *J. Bact.* **9**: 463, 479 and 491, 1924, **10**: 13, 1925.

¹⁰ Churchman, J. W., in Jordan, E. O., and Falk, I. S. *Newer Knowledge of Bacteriology and Immunology*, Chicago, University of Chicago Press, 1928, chap. 3, p. 24.

In order to improve histologic detail further a cytoplasmic stain may be added profitably. A number of these have been tried, including trinitrophenol, eosin, orange G, metanil yellow, acid yellow G. In general, these may be used either before or after the Gram procedure without markedly interfering with the bacterial and nuclear stains. The choice of cytoplasmic stain is governed by the need for a color value which gives maximum contrast between cytoplasm, bacteria and nuclei and at the same time is light enough to permit identification of bacteria when very few are present. For this purpose, trinitrophenol has been found most satisfactory.

The details of the procedure of staining which has given the best results with a variety of tissues follow:

Paraffin sections are brought through xylene and alcohol to water in the usual way:

- 1 Stain with carbol gentian violet for two minutes
- 2 Drain but do not wash
- 3 Apply iodine (I_2 KI H_2O —1 2 300) for one minute
- 4 Apply acetone until no more color is removed (about from ten to fifteen seconds)
- 5 Wash in water. Do not allow the section to dry
- 6 Apply 0.05 per cent basic fuchsin in five hundredth-normal hydrochloric acid for three minutes
- 7 Drain but do not wash
- 8 Apply saturated aqueous trinitrophenol for from thirty seconds to one minute
- 9 Wash in water
- 10 Differentiate and dehydrate in acetone for from ten to fifteen seconds
- 11 Clear in xylene
- 12 Mount in balsam

The gentian violet used is a product of the National Aniline and Chemical Company labeled "Gentian Violet (Crystal Violet)." Its dye content is 90 per cent. The color index number is 681. It has been certified by the Commission on Standardization of Biological Stains with certification number N C 14. The stain was prepared by triturating in a mortar 1 Gm. of gentian violet with 1 Gm. of phenol crystals. Ten cubic centimeters of absolute alcohol was then added. This stock solution was diluted ten times with distilled water, allowed to stand forty-eight hours and filtered before using.

This particular brand of dye is not essential to the method. Equally good results have been obtained using methyl violet 5 B Grubler and crystal violet Grubler.

The basic fuchsin employed is a product of Grubler. Further information about this dye is not available, nor is it known whether the dye is the chloride or the acetate. The important point in its preparation is that it is made up by dissolving 0.05 per cent of the dry dye in a solution having a p_H between 2 and 3. In fact, this is the essential feature of the entire procedure. Whether this p_H is obtained by the use of five hundredth-normal hydrochloric or tenth-

normal acetic acid or a buffered solution seems to be unimportant. This observation indicates that it is immaterial whether the basic fuchsin is a chloride or an acetate. Several samples of basic fuchsin of unknown origin have given almost equally good results. Safranin O Grubler has also been employed successfully at p_H 2.5, but cleaner histologic detail is obtained by the use of basic fuchsin. The most suitable concentration seems to be 0.05 per cent.

The use of a staining rack and dropping bottles rather than staining jars is recommended since it permits the use of uncontaminated reagents.

Preliminary fixation of tissue can be accomplished by the common fixatives, formaldehyde solution, U. S. P. (1:10), Zenker's fluid, Zenker's fluid plus dilute solution of formaldehyde or Bouin's fixative can be used with success. Better histologic detail seems to be obtained by the use of Zenker's fixative without acetic acid.

When viewed under the low power of the microscope the tissue is lightly stained. Gram-positive bacteria are deep violet and easily identified with the low power lens. Where cellular exudation is not too abundant, the deep red gram-negative bacteria can also be seen under the low power lens in contrast to the lighter red nuclei. Under oil immersion both types are sharply differentiated.

Nuclear chromatin stains red, cytoplasm, faint yellow. The nuclei of nerve cells show differentiation of nucleoli and Nissl bodies. Axons are unstained. Myelin sheaths are violet. Edema fluid, fibrin and the fibers of connective tissue are pale pink. Erythrocytes are yellow.

Gram-positive bacteria are deep violet. Staphylococci, streptococci, the mycelia of actinomycetes and the vegetative forms of spore-bearing species are solidly stained. Diphtheria bacilli show barred and granular forms. Human tubercle bacilli are less deeply stained than other gram-positive organisms and show distinct beading.

Gram-negative bacteria are uniformly red. The clubs of actinomycetes are also red and contrast sharply with the gram-positive mycelium.

The chief advantage of this stain is that it not only provides simultaneously good histologic and bacterial differentiation but stains all bacteria that may be present. This is particularly useful in the study of tuberculous tissue where very young tubercle bacilli may not be acid-fast, and secondary invaders are easily identified.

SUMMARY

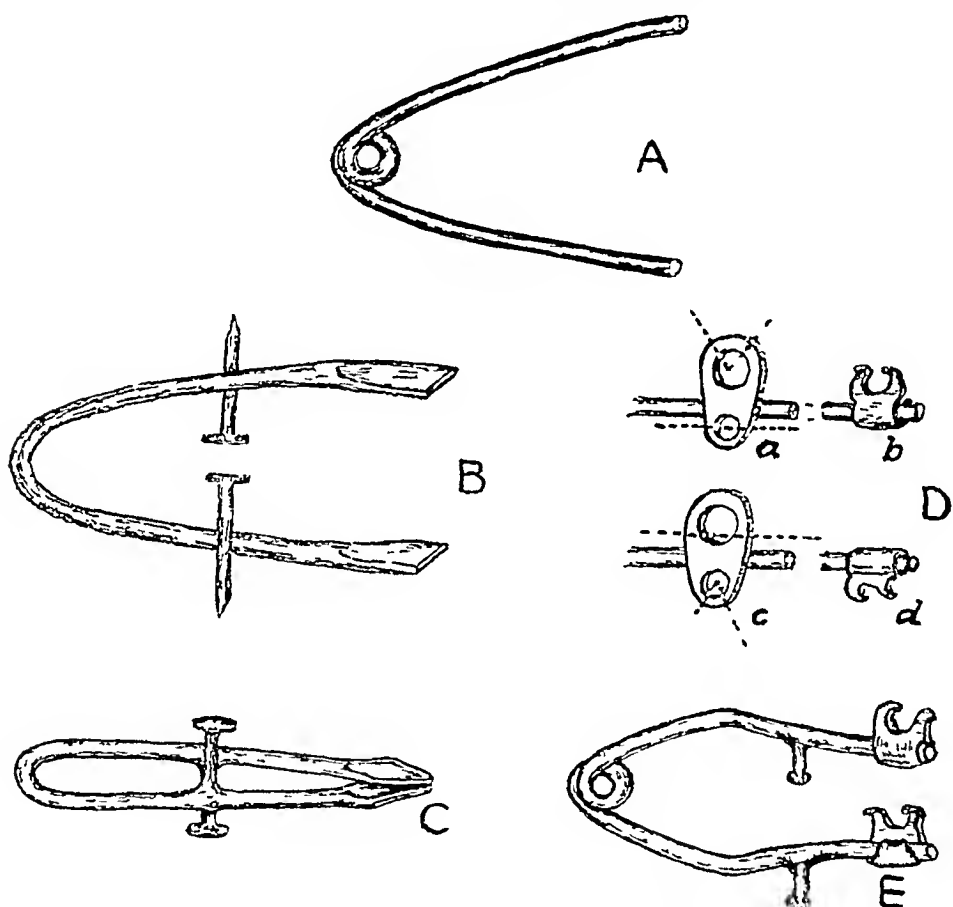
A modification of the Gram stain applicable to paraffin sections is described. The essential feature of the procedure is the use of basic fuchsin at a p_H of from 2 to 3. Good histologic differentiation as well as simultaneous staining of gram-positive and gram-negative bacteria is obtained.

FORCEPS AND RETRACTORS MADE FROM SAFETY-PINS

PAUL GROSS, M D, AND FRANK B COOPER, M S, PITTSBURGH

In the absence of sufficiently small yet accurate forceps, safety-pins may be converted into these instruments with little effort and no expense

The head of a no 3 safety-pin, together with a corresponding amount of the pointed end, is clipped off (figure, *A*) The spiral in the spring is converted into



Drawings illustrating conversion of a safety-pin into a forceps and a retractor

a simple loop by appropriate bending, and the ends of the pin are hammered flat. Small nails of the size indicated (no 18 wire nails $\frac{3}{4}$ inch [19 cm] long) are soldered in position as shown in the figure, *B*. The projecting points of the nails are clipped off, and the cut ends as well as the excess solder are filed smooth. A small amount of solder is spread on the inner aspect of the flattened ends, and the two limbs of the forceps are approximated to the desired position. The jaws of the forceps are then dressed with a breaker-point file so that they will grasp a hair tenaciously (figure, *C*). The tension of the jaws can be increased by filling the loop spring with solder.

From the Institute of Pathology of Western Pennsylvania Hospital, Dr Ralph Mellon, director

A second very useful instrument made by modifying a safety-pin is a self-retaining retractor. For this purpose soldering lugs¹ of the size and shape indicated are employed. Depending on which end of these lugs is used, large-pronged (figure, *Db*) or small-pronged (figure, *Dd*) retractors are fashioned. Cuts are made into the lugs as shown by the broken lines in the figure, *Da* or *c*. These cut lugs are then soldered to the limbs of the safety-pin (figure *Db* or *Dd*, also *E*). The limbs of the retractor and the prongs are bent to give the desired tension. Wire brads are soldered to the limbs of the retractor to facilitate handling. The projecting points of the brads are clipped off, and the joints are filed smooth (figure, *E*).

1 Soldering lugs are obtainable in radio supply stores.

General Review

ETIOLOGY OF AGRANULOCYTOSIS

FREDERICK STENN, M D

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Though agranulocytosis owes its first accurate description to Brown and Ophuls, who reported a case in 1902, there exists information which indicates that this disease was possibly known a century ago and perhaps earlier than that. The blood count and the differential smear were available to physicians hardly much before the opening of the twentieth century, and thus a positive diagnosis of this disease entity was impossible. However, there are clearly described cases of a symptom picture which intimately parallels the modern conception of agranulocytosis, a fact appreciated in 1931 by Pepper. Almost twenty-three hundred years ago Hippocrates recognized a rapidly fatal pharyngeal disease characterized by gangrene with overwhelming symptoms. Celsus and Aretaeus noted the throat with its black eschar, Caelius Aurelianus and Aetius referred to the rare "synanche," the phlegmonous state of strangling of the throat, and Avicenna and Albucasis alluded to it in their memoirs. After their writings were published various names were applied to this condition, and among the common ones were *ulcus syriacum* ou *egyptiacum*, *carbunculus anginosus morbus puerorum*, *gai otillo*, *morbus suffocans*, *morbus strangulatorius*, *ulcère gangi éneux*, *mal de gorge gangi éneux*, *pharyngitis acuta infectiosa phlegmonosa* and *angina maligna*.

In 1857 Adolphe Gubler described the case of a maid who became suddenly ill with a violent sore throat, followed by extensive gangrene of the pharynx, which terminated fatally. In 1859 Ferrie became interested in the new disease to which his groom succumbed with surprising rapidity. In 1860 Musset reported a similar case but with recovery. And in 1861 Trousseau wrote "Besides secondary gangrene there is a primary gangrene that should be regarded as a disease having as its fundamental character necrosis of the mucous membrane of the pharynx and sometimes necrosis of the cheeks and lips," which is "unrelated to any preceding disease, unassociated with epidemics of diphtheria, attacking healthy vigorous subjects without cause and often producing death with variable rapidity." The entity of putrid sore

throat (angina putris) was defined in 1880 by Mackenzie in his treatise entitled "Diseases of the Pharynx, Larynx, and Trachea," as a "primitive gangrene of the pharyngeal mucous membrane, constituting an infection per se, originating independently of any other malady, such as diphtheria, scarlet fever, etc.," always appearing "to be the result of blood poisoning" and commencing "as a severe inflammation which quickly leads to gangrene," the patient suffering from foul breath and extreme prostration and dying in syncope. Of his 5 patients only 2 recovered. It is interesting that Mackenzie observed that "putrid sore throat is only a local manifestation of a profound blood poisoning" and that it has its greatest occurrence "in physicians' nurses and students," facts that are accepted today.

Much of the literature on putrid sore throat, otherwise called acute infectious phlegmon of the pharynx before 1900, referred to the 4 case reports of Senator, who in 1888 called attention to a "little recognized disease neglected clinically and concerning itself with a gangrenous and often fatal illness." Again, in 1889, Maurin undertook a detailed study of "l'angine gangreneuse primitive," collecting reports of 10 cases 4 of which were fatal. His observations agreed with those of Blumenau, who in 1896 declared that the disease appears most frequently among adults, producing a circumscribed or a diffuse form of gangrene in the throat, beginning with general malaise, depression, severe chills with high fever, severe pain in the throat, headache, prostration and often hemorrhages in the nose and throat and terminating with death due to septic poisoning.

However, the earliest accurate report of agranulocytosis was submitted in 1902 by Brown and Ophuls, who described the case of a mother aged 29. Three weeks after operative repair of the vagina and cervix there developed a chill, a temperature of 103 F and a pulse rate of 112, and the patient complained of sore throat. The white blood cells numbered 1,200, with 1 per cent polymorphonuclears. The day before death the leukocyte count fell to 260. The authors entitled the disease acute primary infectious pharyngitis.

Two years later Schwartz described the case of a 9 year old boy with an abscess of the kidney which ruptured into the urinary tract. The leukocyte count was 600, no neutrophils were present.

In 1907 Turk treated a 45 year old woman who was suffering from staphylococcal sepsis and had chills, headache and diarrhea. The tonsils were covered with white exudate. After spending a whole day and night searching over a blood smear he was astonished at his inability to find a single neutrophil.

Baldridge and Needles found in the records of the University Hospital of Iowa City a report of the case of a girl aged 8 years who

died in 1910 of typical agranulocytosis. In the same year Leale reported a case occurring in an infant suffering from marasmus and furunculosis. This case will be referred to later under the title cyclic agranulocytosis.

In 1912 Stursberg reported the case of a man aged 41 with osteomyelitis of one arm who bled from the nose and mouth and complained of oral pain and dysphagia. He died shortly after the leukocyte count fell to 900, with complete absence of granulocytes.

The study of the disease received its greatest impulse and widespread recognition in 1922 after Werner Schultz had reported a group of cases in women between the ages of 18 and 65 years, all of whom died. The clinical course lasted for three or four days in some instances and for one or two weeks in others. The illness began with high fever, with or without icterus, and all the patients showed necrotizing processes in the tonsils, pharynx, tongue, larynx, uvula, soft palate or gums. One patient showed gangrenous colitis. There was no generalized lymphadenopathy or evident hemorrhagic diathesis. The highest leukocyte count was 1,800, and the blood smear showed only lymphocytes and monocytes, with occasional neutrophils. The red blood cell and the platelet count were normal.

Rapidly the medical world became aware of agranulocytosis and articles were published by Leon, Friedemann, Bantz, David, Ehrmann and Preuss, Elkeles, Feer, Lauter and Zadek, in Germany, Lovett, Hill, Hunter, Cannon, Skiles, Hart and Piette in the United States, Flandin, Mouzon, Roch and Mozer in France, Alagna and Gamma in Italy, and Pelnáň in Czechoslovakia. Whether this sudden prominence of agranulocytic angina was due to the watchfulness of the physicians for the condition or to an actual outbreak of the disease is difficult to conclude. Certainly before 1922 the disease was seen rarely. Its frequency after that year has been attributed by some to the extensive therapeutic use of arsphenamine, gold, bismuth or aminopyrine, by some to factors in civilized life that are little understood and by others to a spontaneous outbreak, like a severe epidemic of influenza.

INCIDENCE AND PREDISPOSING ETIOLOGIC FACTORS

Many cases reported in the literature as instances of agranulocytosis were confused with those of aplastic anemia, aleukemic leukemia, overwhelming sepsis and other diseases, so the number of cases has been somewhat exaggerated, though the fact is to be heeded that this disease has been overlooked until recently and that probably its great incidence is due to its better recognition. Today the United States has the highest incidence, according to the Department of Vital Statistics at Washington, 1,981 deaths were reported in 1931, 1932, 1933 and 1934, with

the greatest frequency in California, New York, Illinois and Pennsylvania. Roberts and Kracke expressed the belief that the actual incidence reached many thousands. Germany and Austria, where the first reports

TABLE 1—Deaths in the United States from Agranulocytosis*

1931	1932	1933	1934	Total
421	450	572	538	1,981

Data supplied by the Department of Vital Statistics, Washington, D. C.

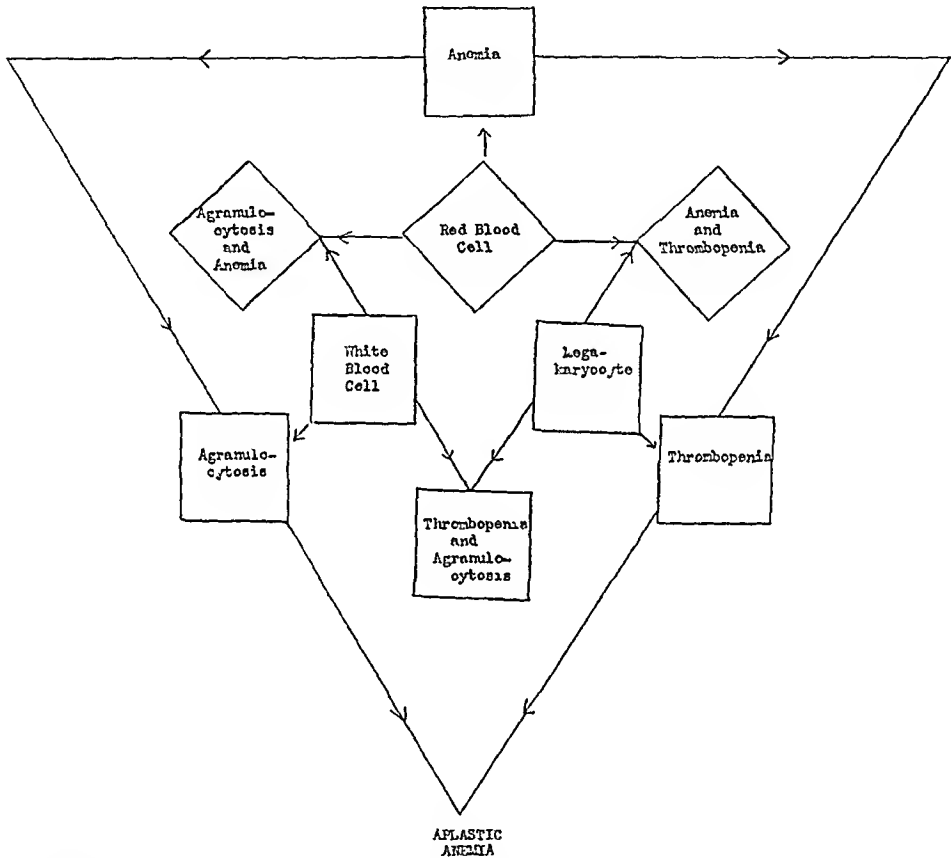
TABLE 2—Incidence of Agranulocytosis in the United States Between 1931 and 1933*

	Agranulocytosis			Agranulocytic Angina		
	1933	1932	1931	1933	1932	1931
United States	82	84	96	400	366	325
Alabama		1	1	6	3	2
Arizona	1			1	4	
Arkansas				2	2	4
California	3	6	5	40	16	29
Colorado		2		4	5	2
Connecticut	2	4	2	9	9	8
Delaware					1	2
District of Columbia	1			3	6	1
Florida	2	2	2	5	2	
Georgia	2	2	8	6	2	4
Idaho			1	1	1	1
Illinois	6	8	10	41	28	24
Indiana	2	2		9	7	5
Iowa	3	3		8	12	11
Kansas	2	3	1	8	4	5
Kentucky	5	3	1	6	2	1
Louisiana		2	4	4	9	8
Maine			1	2		
Maryland	3		1	10	2	5
Massachusetts	2	2	6	31	15	14
Michigan	4	5	8	12	23	17
Minnesota	1	1	3	10	9	9
Mississippi	1		1			1
Missouri	2	1	4	9	9	14
Montana		1	1	3	1	3
Nebraska	1	4	3	11	10	5
Nevada						
New Hampshire				1		
New Jersey	2	4	3	24	18	15
New Mexico						
New York	6	3	9	94	74	71
North Carolina	2			4	6	2
North Dakota	2	1	1		1	1
Ohio	5	6	2	15	10	16
Oklahoma	3	1		2	6	3
Oregon		1		4	2	1
Pennsylvania	6	3	4	40	23	15
Rhode Island	1	2	1	5	1	2
South Carolina		2		4	3	
South Dakota					1	
Tennessee	3		3	3	5	5
Texas	5	2	4	14	6	6
Utah				2	1	
Vermont					1	
Virginia					1	
Washington	2	2	1	12	8	4
West Virginia		1	1	9	5	2
Wisconsin	1	1	1	5	3	2
Wyoming		2	3	10	11	5
Alaska		1				
Hawaii						
Puerto Rico						

* The terms agranulocytosis and agranulocytic angina are headings under which physicians reported the disease. Data published by the United States Bureau of the Census, Washington, D. C.

50 and 60 Ridpath noted that in 80 per cent of all cases reported in women the disease has developed within the menstrual age, under 45 years, and that the symptoms have occurred one or two days after the onset of catamenia. In most of the cases reported in children the condition either has fallen into the category of aplastic anemia or has been precipitated by severe sepsis or the taking of a drug.

Seldom encountered in the Negro and yellow races, agranulocytosis is confined to Caucasians. The rarity of cases in Negroes was observed by Kracke and Parker, who stated that over a five year period at the



Bone marrow insufficiency. A schematic chart illustrating the interrelationships of anemia, thrombopenia, agranulocytosis and aplastic anemia.

Emory University Division of the Atlantic City Hospital, which has an annual admission of 75,000 Negroes, not a single case of idiopathic agranulocytosis was seen. No nationality is exempt.

Persons with moderate means and wealthy persons are more often attacked than the poor. This difference in social status may be only apparent, for those in comfortable circumstances are more likely to seek good medical advice early. Reports from the various charity hospitals reveal the infrequent occurrence. Dr Karl Meyer stated that at the Cook County Hospital Chicago, 9 cases of agranulocytosis

occurred between the years 1922 and 1935, only a few of them being of the idiopathic type. A study of the occupations concerned shows that the housewife is most frequently affected, then the physician, the nurse, the maid in the hospital, the medical student, the hospital orderly and the laboratory technician and finally the physician's relatives and the school teacher. The farmer, business man, clerk, tailor, dentist and others are less likely to be subject to the disease. Kracke and Parker emphasized its occurrence "fifty times more frequently in physicians than lawyers, and 200 times more frequently in nurses than in female school teachers." This peculiar tendency to affect those engaged about a hospital has been appreciated by Stellhorn and Amolsch, Harkins, Conner and his associates, Hinton, Sydenstricker, Fitz-Hugh and Comroe and Kracke.

Most authors have expressed the opinion that the seasonal incidence is unimportant, though Lichtenstein has observed most of his cases in winter.

No epidemiologic factors have been observed.

Only rare case reports mention any congenital or familial tendency. Wolff saw two sisters who took ill at one time with angina, a brother had agranulocytosis and a sister had myeloid leukemia. Hart and Strasser each observed the disease in two sisters. Roberts reported the case of a woman who died of myeloid leukemia. Her son had a leukocyte count of 3,500, and her sister became ill with agranulocytosis. Burcky noted the case of a girl who had a leukocyte count of 3,800, with 12 per cent granulocytes, following a sore throat from which she recovered. Ten months later her mother died of typical agranulocytosis.

The theory that a tendency toward leukopenia exists in a certain proportion of society and that it may be a decisive factor in precipitating this disease process is based on the analysis of large numbers of blood counts. Feer studied the case records of 29,498 patients between 1915 and 1925 and found that 193 had leukocyte counts below 3,000. In a study of 8,000 case records of patients seen in private practice in the South, Roberts and Kracke noted that one fourth of the patients and one half of the women between 40 and 60 years of age suffered from mild granulopenia. Mettier and Olsan stated that at the University of California Hospital 11.6 per cent of 10,000 case records from 1920 to 1931 revealed that the patients had leukopenia, 51.4 per cent of the patients with leukopenia were women, and 47.6 per cent were men. Despite this wide statistical discrepancy, these data teach that chronic leukopenia exists and that a leukopenic predisposition when mild, according to Cabot, Roberts and Kracke and Rosenthal, leads to vague symptoms, such as chronic fatigue, malaise, anorexia, neurasthenia, psychosis and exhaustion. When it is more severe granulopenia

is succeeded by the more drastic symptoms of agranulocytosis, as suggested by Roberts and Kracke. Doan observed that when infection develops in a person with chronic leukopenia, leukocytosis results and produces a response in the bone marrow, but in those persons who show symptoms of malaise and fatigue, with relative lymphocytosis, absolute neutropenia and a shift to the left, infection produces a more severe leukopenia and renders the myeloid tissue insufficient. Hueber studied 79 patients with agranulocytosis, 25 of whom exhibited fatigue extending for from four weeks to one year preceding the attack. These data suggest that a person with chronic leukopenia is particularly likely to have an acute attack of agranulocytosis.

Inquiry into the previous history of patients with this disease gives rise to numerous suggestions, but no single factor is constantly present. In Hueber's review of 79 cases, 49 patients had recently had injuries, operations or infections. Kastlin's review of 43 cases showed 27 with no history of any previous infection, while in the others there was a history of typhus, tuberculosis, syphilis, pneumonia, anemia, infection or operative procedure. Zetterqvist, Babbitt and Fitz-Hugh, Ridpath, Reznikoff, Hanzlik, Kracke and others reported cases in which the disease was precipitated by the extraction of teeth. In other cases it has followed a major operative procedure, such as thyroidectomy, or a fracture. Not infrequently the attack begins after a debilitating disease, especially influenza, as pointed out by Hirsch, Korach, Ottenheimer, Roberts and Kracke and others. Continued exhaustive states, toil, worry, asthenia and senile states in asthenic women have been mentioned as possible factors, in addition to arthritis and treatment for pyorrhea. Prolonged anemia has been stated to lead to dysfunction of the myelocytic tissue. Exposures of the skin to ultraviolet or infra-red rays, sunlight and x-rays are listed as factors. Some writers have blamed dietetic "obesity cures."

BACTERIOLOGY

No bacterium, group of bacteria, toxin or filterable virus has been demonstrated to be the causative factor of agranulocytosis. The investigations of Schultz, Friedemann, Licht and Hartmann, Zetterqvist, Roberts and Kracke, Harkins and others have shown that these factors are secondary. Examination of the lesions of the throat, gastro-intestinal tract, vagina, skin or some other location has consistently shown the presence of numerous organisms. *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus viridans*, *Streptococcus haemolyticus*, Vincent's spirochetes and fusiform bacilli, *Bacillus diphtheriae* diphtheroid forms, *Bacillus pyocyaneus* and various types of spirilla and pneumococci, such as are found in ulcerative processes of many diseases. Werner Schultz injected into rabbits tonsillar tissue of patients dying of

agranulocytosis, Weismüller injected secretions from the throat intraperitoneally into guinea-pigs, and Lichtenstein performed similar experiments, with negative results in each case. In like manner, blood culture has proved fruitless. The organisms isolated have been *Str. haemolyticus*, *Str. viridans*, *Staph. aureus*, *Staph. albus*, Friedländer's bacillus, pneumococci of types I to IV, *B. pyocyaneus*, *Bacillus typhosus*, *Bacillus paratyphosus*, *Bacillus coli*, Vincent's organisms, malarial parasites, *B. diphtheriae*, meningococci, *Streptothrix* and others. In their review of the literature Kracke and Parker found reports of 74 blood cultures that gave positive results, and in 395 instances either the cultures gave negative results or the results were not mentioned. The cultures gave positive results in 75 per cent of the cases in which they were taken, and the streptococcus was the commonest invader. Stocke noted that in only 23 per cent of 88 cases did the blood cultures show positive results, and in many of these the cultures were made post mortem. In Lichtenstein's 27 cases 13 of the blood cultures showed negative results, and 4 showed *Str. haemolyticus*. In Kastlin's review of 43 cases 9 blood cultures gave positive results, the organisms isolated being *Str. haemolyticus*, *Staphylococcus*, *B. pyocyaneus* and *B. coli*. Many patients die without having any organisms demonstrable in the blood.

Failure to produce the disease experimentally was reported by Garrod and Williams, who injected intravenously into rabbits and intraperitoneally into a guinea-pig a sample of blood of a patient dying of agranulocytosis. Schultz and Jacobowitz used rabbits, Licht and Hartmann, rabbits and white mice, Weismüller, guinea-pigs, and Bix, guinea-pigs and white mice, and all failed to precipitate the disease. Roberts and Kracke fared similarly after giving rabbits and guinea-pigs intravenously, subcutaneously and orally *Str. haemolyticus* obtained by culture of the blood of a patient dying of the disease.

Some investigators, however, have obtained a decided leukopenia experimentally. Green inoculated a guinea-pig with gram-negative bacilli obtained from a patient's blood and necrotic tissue from a patient's mouth and succeeded in obtaining ulceration of the gastro-intestinal tract, oral cavity, uvula, vagina, cervix and rectum, bronchopneumonia, submural and subpericardial hemorrhages, and depression of leukopoiesis. Schattenberg and Harris produced depression of the leukocyte and granulocyte count in rabbits with *Staph. aureus* isolated from the blood of a patient and with the toxins of *Bacillus enteritidis*, *Str. haemolyticus* and *Bacillus Welchii* isolated from the stool of a patient. Lichtenstein injected intravenously into each of 6 rabbits 1,000,000,000 streptococci obtained in pure culture from a patient's blood and produced marked leukopenia. He expressed the belief that the streptococci have a specific effect on the bone marrow. *B. pyocyaneus* has been

thought by some to be a possible etiologic agent. In 1924 Beatrice Lovett cultured this organism from the throat and the vagina of a patient dying of the disease and on injecting it into a guinea-pig obtained mild leukopenia and neutropenia. Skiles, Linthicum, Rose and House, Kastlin and others have found it to be an organism common in this disease. Friedemann obtained it from the heart, liver, and spleen and once from the blood, and Stillman reported it in the blood of 2 patients at autopsy. That *B. pyocyaneus* liberates a powerful leukocidin was shown in 1889 by Gheorghiewsky, who injected *B. pyocyaneus* into the peritoneal cavity of the guinea-pig and found marked degenerative and lytic changes in the neutrophils contained in the exudate. Yet Kracke was unable to reproduce the disease after injecting into animals *B. pyocyaneus* cultured from material from ulcers of the mouth and blood in 2 cases, and similar failures have been reported by others.

Fried and Dameshek observed that a relatively small intravenous dose of *Salmonella supestifer* produced slight leukopenia, a large dose produced greater leukopenia, and an overwhelming dose produced areas of necrosis in the bone marrow.

Meyer and Thewlis and Kracke were unable to verify the results obtained by Dennis that chronic and acute granulocytopenia can be produced when capsules containing broth cultures of pyogenic organisms are placed within the abdomen of rabbits. Meyer and Thewlis using 19 rabbits were unable to produce leukopenia or agranulocytosis by this method, with the exception that *B. pyocyaneus* caused acute leukopenia without a significant decrease in the percentage of neutrophils. Nor were Piersol and Steinfield able to produce long continued granulopenia in healthy rabbits by using the common bacteria.

This experimental work is interesting in view of the studies of C. W. Wells, who injected into rabbits huge doses of streptococci, staphylococci and typhoid bacilli and obtained an initial leukopenia followed by leukocytosis. Using pneumococci of high virulence, Long obtained similar results. In addition, Wells noted that when dead bacteria were injected into rabbits there followed a marked leukopenia in the peripheral blood and that during this peripheral leukopenia the blood obtained from the spleen and the hepatic parenchyma contained enormous numbers of leukocytes, a fact confirmed by Goldscheider and Jacobs. Thus, such bacteriologic data as relate to the etiology of agranulocytosis must be accepted with caution.

The symbiosis of several bacteria has been suggested as the etiologic agent, but proof is lacking.

In 1925 Schultz suggested the possibility that the toxic action of a filtrable virus has a special affinity for the myeloid tissue. As yet such a virus has not been demonstrated.

Typhoid—In several cases of agranulocytosis that have followed typhoid prophylaxis (Kracke, Schur and Bromberg and Murphy) the condition has been thought to be due to an overwhelming foreign protein reaction in a sensitive person

PATHOLOGIC ANATOMY

It is generally conceded that the essential pathologic feature of agranulocytosis is concerned with a severe degeneration of the granulocytic system associated with normal or nearly normal megakaryocytic and erythroblastic elements. Most frequently the bone marrow is hypoplastic or aplastic. The myeloblasts and myelocytes undergo pyknosis, nongranulation, vacuolation and hyalinization. These observations have been submitted by Koch, Pett, Uffenorde, Jackson, Zikowsky, Licht and Hartmann, Oppikoffel, Dameshek and others. Similar degenerative changes are observed in the myeloid elements in the less frequently reported cases in which the marrow is hyperplastic (Fitz-Hugh and Kiumbhaar, Dameshek and Ingall, Baldrige and Needles, Buck, Fitz-Hugh and Connroe, Jaffe, Mettier and Olsan, David and Rose and House) Jaffé summarized these observations as follows

In both hyperplastic and the non-hyperplastic bone marrow the granulopoietic cells revealed severe degenerative changes. I have obtained the impression that the specific granulation is the first to become affected while the nucleus remains intact for some time and may even divide by mitosis. The specific granules with all their outlines become indistinct and small vacuoles often appear around them. The granules later dissolve into the vacuoles and pale purplish-pink droplets result which fuse together giving a vacuolated appearance to the cells. In the meantime the chromatin has become separated into coarse, sharply defined clumps and the nuclei have disappeared. An occasional mitosis may be detected in a cell which has been deprived of its granulation. The mitoses are, however, atypical, with short and clumsy chromosomes, and I think they do not pass beyond the metaphase. Finally, the nucleus shrinks, the cytoplasm coagulates, and the cell is dead. With the dissolution of the granules into the vacuoles the oxidase reaction becomes negative.

By virtue of these data it is assumed that some factor must be operative which either causes a cessation in the development of the white blood cells or prevents their migration into the blood stream. The cases of the hyperplastic type have been explained as due to a blockade which prevents the entrance into the blood stream of the mature leukocytes, and those of the aplastic type, by long continued blockade which leads to degeneration and destruction of the parent cells of the neutrophils.

Though anemia is frequently observed, especially in the prolonged cases, erythropoiesis proceeds normally, the lymphocytes, plasma cells and reticulohistiocytes of the bone marrow are normal but as Rose and

Houser, Haitwich, Van der Wielen and Jaffe have recorded, degenerative changes are often observed in the megakaryocytes, a fact that may explain the frequency of hemorrhagic diathesis. The giant cells are increased in number.

The ulcerations that occur in the oral cavity, tonsils, palatal region, larynx, stomach, anus, vagina and skin show extensive necrosis penetrating into the deeper tissues, with marked hemorrhages and numerous hyaline thrombi, associated with bacteria collected in huge numbers. Plasma cells are grouped along the border of the ulcer, in which is a diffuse lymphocytic infiltration but no neutrophils. The lungs often show confluent bronchopneumonia, with little evidence of inflammatory cellular reaction, subpleural hemorrhages are frequent, pleurisy with effusion is occasional. The pericardium displays many petechiae and ecchymoses, and the heart shows, like the liver and kidneys, parenchymatous degeneration. In the liver necrotic foci occur, and the Kupffer cells are swollen. The spleen is swollen and pulpy and rich in histiocytes and plasma cells. The kidneys show hyaline thrombi in the medulla and petechial hemorrhages. The lymph nodes show petechial hemorrhages. The reticulo-endothelial apparatus is proliferated not only in the bone marrow but also in the spleen, liver and lymph nodes. Rose and Houser and Rotter noted widespread necrosis and thrombosis of arterioles and venules.

MECHANISM

Severe reduction or absence of the neutrophils has been explained by three hypotheses: (a) destruction in the peripheral blood stream, (b) migration to various branches of the blood stream and to the viscera and (c) failure either of maturation of the myeloid elements in the bone marrow or of discharge of the mature granulocytes from the marrow into the peripheral blood stream. No convincing information has as yet been supplied to support the theory of destruction of the granulocytes in the peripheral blood stream. Though Brown found an increased amount of uric acid in the urine in his case, examination of the urine for this constituent has been generally neglected. No changes are observed in the white cell count or smear when the patient's blood is mixed with normal blood, and the injection of granulocytes from the patient's blood into animals produces no effect. The idea of an abnormal distribution of the neutrophils has little support. Studies of the numbers of the white blood cells and the neutrophils in capillary, venous and arterial blood in patients display no appreciable numerical differences. In addition, the significant feature in the pathologic picture of this disease is the unusual diminution or absence of the neutrophil in every organ and tissue.

Generally accepted, however, is the hypothesis that the disease process, whether bacteriologic, pharmacologic, endocrine, nutritional or of some other type, strikes as the primary and essential focus the myeloid elements of the bone marrow. The bone marrow is to be regarded, Doan has aptly suggested, as an organ subject to the same pathologic and physiologic changes as any other—hyperplasia and hypoplasia, sufficiency and insufficiency. What factors are responsible for these variations is only too little known. Lescher and Hubble supposed the existence of specific regulating factors for each of the cells of the bone marrow, and when one of these factors is deficient there results the disease characterized by this deficiency—agranulocytosis or thrombocytopenia or both, or even aplastic anemia when all are deficient. Beck expressed the belief that the essential pathologic change lies in the absence of a maturation factor for the neutrophils, and Fitz-Hugh and Krumbhaar said in this regard

It would seem probable that a maturation factor is at work either arresting development of white cells in their formative centers or producing degenerative changes in them before sufficient development for normal migration into the blood stream, or possibly a combination of both factors. This hypothetical factor would conceivably check the granular series of blood cells at the myeloblast-myelocyte stage in the bone marrow and the lymphocyte series at or near the lymphoblast stage in the spleen and the lymph nodes. Viewed from this standpoint the analogies to pernicious anemia are obvious. Pernicious anemia too has remissions and relapses. It, too, is as yet an idiopathic disorder with probably a constitutional background of prime importance. Its relapses are characterized by megaloblastic hyperplasia of the bone marrow caused by some maturation inhibiting factor, directed against the red cell series.

Doan suggested the possible importance of the lack of a chemotactic factor and pointed out that nucleic acid and its decomposition products stimulate the bone marrow to pour its myeloid elements into the peripheral blood stream. He observed that when administered to laboratory animals these substances caused myeloid hyperplasia in the bone marrow and a marked increase in the white blood cell count. He stated

Nucleic acid and its degradation products exert a chemotactic effect on normal foci with a prompt effective increase in the delivery of granular leucocytes to the peripheral circulation under a controlled physiologic or rhythmic mechanism.

A short course of injections stimulates a myeloid hyperplasia of normal marrow without otherwise injurious consequences, which is reflected by a relative and absolute increase in the amphophilic granulocytes in the blood stream of rabbits.

When the neutrophils break down, nucleic acid products are formed, and these stimulate the myeloid tissue, whatever the cause of neutropenia in agranulocytosis, these products are greatly diminished in the blood stream.

Besides maturation, regulation and chemotactic factors, what rôle do vasomotor and nervous activities play? And what are the factors concerned in the delivery of the granulocytes to the circulation? These are basic problems confronting the student of this disease.

What is the relationship between the fall in the number of neutrophils and the onset of the clinical symptoms? Data have accumulated which point convincingly to the conclusion that the myeloid tissue of the bone marrow becomes insufficient whether owing to cryptogenic factors, toxins, bacteria or medicaments, the neutrophils of the peripheral blood are not replaced when they die, and soon the blood shows an alarming scarcity or absence of these elements, permitting the entrance into the blood of bacteria at vulnerable points, creating septicemia, which is fatal. Schultz, Friedemann, Meyer, Carter and Domarus reported cases in which angina was distinctly secondary, and Kommerell, Reye and Bock and Wiede noted the typical blood picture before the onset of the symptoms. These observations were confirmed by Hunter, Bantz, Ehrmann and Pieuss, Rutledge, Hansen-Pruss and Thayer, and Lauter, Leon and Lindbloom. Rutledge, Hansen-Pruss and Thayer described a remarkable case of cyclic agranulocytosis beginning in a boy at the age of $2\frac{1}{2}$ months and occurring every twenty-one days for twenty successive years. Preceding each attack the neutrophil and white blood cell count underwent a distinct fall. Jackson and Parker reported the case of a patient with a white blood cell count of 500 before the appearance of any clinical symptoms or signs, and on the fifth day there was a prompt rise in temperature, with chills, headache, prostration and sore throat. Roberts and Kiacke had an unusual opportunity to follow in detail a single case illustrating the sequence of events. In a woman aged 72 with a normal blood picture there suddenly developed weakness, exhaustion, stupor, fever and rapid pulse. The white blood cell count was 2,050, and no polymorphonuclears were present. Though necrosis of the tongue developed, she recovered and became normal clinically and hematologically in two months. After that, however, a second attack developed in which the white blood cell count fell, and the neutrophils disappeared completely, but the patient was clinically well. It was only after four days of complete agranulocytosis that there developed symptoms of stupor, necrosis of the tongue and then pneumonia and finally death. Abrahams, however, described a case of pure agranulocytosis which persisted for fifteen days without the development of sepsis or any more serious manifestation than malaise.

CLINICAL COURSE

Whether of primary or secondary origin, the clinical course of agranulocytosis is always the same, just as the course of pernicious anemia is typical, whether caused by idiopathic factors or by syphilis.

or *Diphyllobothrium latum*. The disease is characterized by an onset of premonitory symptoms, consisting of headache, malaise, weakness and anorexia, followed by ulceration and necrosis in the gastro-intestinal, respiratory or genito-urinary tract or of the skin. Most commonly this gangrenous process develops in the pharyngeal or oral cavity, with dysphagia and sore throat, in the upper part of the larynx, with dyspnea and swelling of the neck, and in the vagina or the anus or on the skin, with local symptoms. Angina is not always present, nor is ulceration. The course is usually stormy, with the temperature ranging between 102 and 105 F, with chills, sweats, delirium and early prostration. A hemorrhagic diathesis and icterus are common. The spleen may be slightly enlarged. Some degree of anemia commonly exists, with alarming neutropenia or complete absence of neutrophils and usually leukopenia. Septicemia follows, and in from 65 to 75 per cent of cases death ensues in from four to six days. Should the myeloid tissue respond, the stage of sepsis is prevented or overcome, and the patient may survive, not infrequently long enough to be precipitated into the throes of another attack, or, as happens less frequently, he may recover completely.¹

Only one feature is essential for the diagnosis—severe neutropenia. But who will say how severe the neutropenia must be for the diagnosis to be established? Primary and secondary agranulocytosis may occur coincident with anemia, thrombopenia or both, and each of these may be slight, moderate or severe. It is often impossible to draw a sharply demarcating line when the hematologic picture merges into the realm of thrombopenia or aplastic anemia.

PRIMARY AGRANULOCYTOSIS

In this study the subject is divided for convenience into primary and secondary agranulocytosis. The primary type refers to that group of cases in which a specific causative agent has not been found. It is probable that future observation and investigation will show this group to be of secondary origin. The secondary type includes the group of cases in which the condition is caused by medicaments, chemicals or sepsis.

The theories that have been advanced to explain the nature of primary or idiopathic agranulocytosis fall into one of the following groups: infections or toxins, aplastic anemia or leukemic and developmental, vitamin, anaphylactic or endocrine factors.

Infection and Toxins—The most popular of these conceptions is the first, supported by Roch and Mozer, Baltzer, Hunter, Weiss, Bock and

¹ An increase in the number of monocytes in the peripheral blood is a favorable prognostic sign (Lichtenstein, Miloslavich and Murphy and Schilling), and a rapid sedimentation rate is an ill omen (Benhamou and Domarus).

Wiede, Versé, Leon, Fitz-Hugh and Comroe, Reye and Wohlwill, Petri, Lovett and many others, who have maintained that the essential process is one of sepsis, either specific or nonspecific, which overwhelms the bone marrow in a susceptible person of low resistance. Some have considered the disease to be analogous to noma of the ancients (Turk, Stuisberg, Marchand, Schultz and Koch), others have believed that it is related to tropical phagedena (Plehn), and still others have believed that it is related to tuberculosis. Some have raised the question of the action of powerful toxins which either paralyze the bone marrow or produce a negative chemotactic effect on the granulocytes of the bone marrow. Occasionally carcinoma and lymphosarcoma produce marked depression of the bone marrow without demonstrable invasion of this organ, leading one to suspect toxins (Piersol and Steinfield). Jacobson offered the conception that the myelocytes, which, according to Sabin, are developed in the open sinusoids, are exposed to high concentrations of toxins carried in the blood that directly bathes these cells.

Aplastic Anemia—In 1925 Frank declared that agranulocytosis is only a part of aleukia or panmyelophthisis or aplastic anemia. Zetterqvist saw truth in this idea, for in many cases, as previously noted, agranulocytosis is associated with anemia and thrombopenia. Duke offered the interesting suggestion that the disease is an atypical aplastic anemia acting selectively on the granulocytic centers, the result being an infection of such great severity that death occurs early without the opportunity for the anemia or thrombopenia of aplastic anemia to develop. In addition, gold, bismuth, arsphenamine and benzene have each produced depression of one, two or all three of the constituents of the bone marrow, indicating possibly a kinship between agranulocytosis, thrombopenia and aplastic anemia. Diguglielmo has entitled the syndrome acute partial aplastic myelosis.

Among those who have expressed the belief that agranulocytosis is a relative of the leukemias are Pelnář, Strumia, Leon, Hirschfeld, Mouzon, Roch and Mozer, Naegeli, Sternberg and Brogsitter and Kress. The literature records cases in which acute leukemia developed in a patient with an agranulocytic blood picture, but these probably best fall into the group of instances of aleukemic leukemia. Jaffé has stated that the disease may be a terminal complication of aplastic anemia, myelogenous and lymphoid leukemia and Hodgkin's disease.

Hypoplasia of Bone Marrow—Many writers have adhered to the theory of hereditary hypoplasia of the bone marrow, an idea first suggested by Turk and later emphasized by Lauter, Ehrmann and Preuss and Feer. Roch and Mozer and Dahlgreen claimed that this abnormality becomes apparent during infection. But agranulocytosis is often seen developing in patients in whom the reaction to infection both

previous and subsequent to the attack of agranulocytosis is normal and, furthermore, nucleic acid injected into such patients who have survived the disease produces a normal neutrophilic response (Lichtenstein)

X Factor —Castle has obtained evidence that some disorder of the stomach plays a part in the etiology of pernicious anemia. Rosenthal has proffered the question as to whether such an unknown factor may be operative in agranulocytosis.

Vitamins, Foods and Minerals —Though close scrutiny of the diets of patients with agranulocytosis fails to disclose any significant data, Miller and Rhoads have succeeded experimentally in producing neutropenia in dogs by feeding them the Goldberger black tongue-producing diet. Attention is also called to carbohydrate, fat, protein and mineral deficiency in foods.²

Anaphylaxis —Nothing can be concluded from the present knowledge of anaphylaxis. Only suggestive data are supplied in the literature (Rutledge, Roberts and Kracke, Baldrige and Needles, Phillips, Baldrige, Hare and Childrey and Fondé). Kommerell has expressed the opinion that bacteria or their toxins sensitize the bone marrow, and the resulting shock is exhibited in a blockade or arrest of maturation in the bone marrow. Pepper asked

Is it possible that an allergic person experiences sharper drops in the leucocyte count physiologically than in the normal subjects and negligible causes bring about an allergic leucopenia? If at the onset of such a reaction a tooth is drawn or some other opportunity for bacterial invasion occurs the picture of Schultz's syndrome may be present. It is possible that in a person who is allergic to certain bacteria a slight sore throat is the starting point for an allergic leucopenia which then permits other bacterial invasions with a development of characteristic non-reacting local lesions.

Endocrine Factors —Very early Friedemann suggested the importance of hormonal factors, as he had been influenced by the favorable effect of irradiation in agranulocytosis. Each of the organs of internal secretion has been incriminated since then, deficiencies of ovarian and pituitary secretion having been particularly mentioned. In 17 of his 18 cases of agranulocytosis occurring in young women, Thompson observed that subjective symptoms occurred within one or two days of the onset of the menstrual period. Six of these patients had one or more recurrences, and each recurrence coincided with the onset of catamenia. Rid-

² John H. Stokes, of Philadelphia, and S. William Becker, of Chicago, have called attention to the frequency of neurodermatitis, a cutaneous eruption occurring in the highly nervous hypersensitive patient and developing under the pressure of physical or mental strain. If disease of the skin may ensue as a result of a neurogenic factor, is it improbable that agranulocytosis may be produced by a similar cause?

path found that 80 per cent of all cases reported have developed within the menstrual age and that the symptoms have appeared one or two days after the onset of catamenia. Jackson and Merrill reported a similar experience. The case of cyclic agranulocytosis reported by Rutledge, Hansen-Pruss and Thayer has shown the absence of female sex and gonadotropic hormones synchronously with the fall in the white blood cell and neutrophil counts.

Sigaard Andersen noted a rise in the granulocyte count after the administration of thyroid, and Lichtenstein, after thymus, some have advocated the use of spleen.

The cases of cyclic agranulocytosis, as reported by Rutledge, Etipol, Doan and others, suggest that the controlling influence may be similar to that of other cyclic conditions, such as hibernation, sleep, estrus and menstruation, which according to Cushing are controlled by the hormones liberated by the diencephalopituitary apparatus.

The administration of yellow bone marrow to patients suffering from agranulocytosis has given gratifying results in some cases. Employed in 1933 by Watkins and Giffin, it has been used by Flipse, of Miami, and others. Marberg, of the Otho S. Sprague Institute, Chicago, has perfected a concentrate of yellow bone marrow which has proved potent. Fenger stated that its greatest worth is appreciated in cases of acute agranulocytosis, in which after its use the neutrophil and white blood cell counts rise sharply.

Beck said

A maturation factor for erythrocytes has been found, it now remains to find a maturation factor for granulocytes. Chemical analysis of bacteria known to cause a sustained leucocytosis, owing to the fact that they supply both a chemotactic and a maturation factor, might lead to a solution. It seems more logical to search within the body for a specific organ extract regulating granulopoiesis. Animal experimentation with the removal of different glands of internal secretion or the destruction of other tissue by various substances with a careful check on the granulocytes might lead to valuable information not only as to etiology but also to treatment.

SECONDARY AGRANULOCYTOSIS

Though the most exacting history obtained from many patients suffering from this disease has revealed no responsible factor, it is felt by some that many of this primary group can be shown to have taken a medicament that is under suspicion.

Secondary agranulocytosis is identical with the primary form in its pathologic picture, symptoms, course, prognosis and terminus. Its characteristic feature is its remarkable rarity, leading to the conclusion that the myeloid tissue in a certain few persons possesses an idiosyncrasy to arsphenamine, bismuth, gold, benzene, di-nitrophenol, aminopyrine, barbital and amytal—substances to which attention is now invited. The

cases in which the condition develops in the course of sepsis also fall in this group

Arsphenamine—Although arsphenamine was introduced into therapeutic use by Paul Ehrlich in 1910, from six to nine years elapsed before attention was focused on the postarsphenamine blood dyscrasias by Evans, Lerride and Labbé and Langlois. These complications are very rare. Loveman collected reports of 64 cases of aplastic anemia, purpura haemorrhagica and agranulocytosis from the literature. Cole and his co-workers observed 2 cases of purpura haemorrhagica among 12,212 patients who received 78,350 injections of various arsenicals over a period of ten years. Aubertin and Lévy found in the literature reports of 28 cases of agranulocytosis following antisypilitic therapy. 13 patients were males and 15 females, the ages varying from 11 to 50 years, the average being between 20 and 40 years. Most of the subjects with this complication are robust and free from any pathologic complication. Postarsphenamine accidents are observed most frequently in women, according to Emile-Weil and Ishwall. The disease appears at ages ranging from infancy (hereditary syphilis) to nineteen years after the appearance of chancre. The type of arsphenamine does not seem to be important, arsphenamine, neoarsphenamine, sulfarsphenamine, silver arsphenamine or other preparations. The doses administered have never been greater than the average. The date of occurrence is variable, in many cases being during the first series of injections but in most instances during the second and third series. The time that elapses from the administration of the last injection to the development of the disease varies from one to eight days, with itching of the skin, headache, sore throat, vomiting, malaise and fever as the earliest symptoms. Many of the patients show early ulcerative necrotic anginal lesions and hemorrhagic manifestations. In some of the cases the picture conforms to Schultz' description, some patients have mild or marked anemia, and still others have anemia with thrombopenia—all grades are encountered merging with aplastic anemia. Dodd and Wilkinson stated that an initial stimulation of the blood-forming elements precedes a subsequent severe depression. The mortality is about 50 per cent. Autopsy reveals a complete or almost complete disappearance of the cells of the myelocytic series, identical to that noted in idiopathic agranulocytosis and benzene poisoning.

Arsphenamine contains the benzene ring, but there is no evidence that it breaks down into benzene in the body. According to Cushny, when arsphenamine is injected intramuscularly or subcutaneously, arsenic appears in the urine and stools, disappearing from the urine in from ten to fourteen days and from the stools later. When injected intravenously it appears unchanged in the urine in from five to ten minutes and persists in this form for five or six hours, thereafter arsenites and

arsenates are found in the urine for several days and in the stools for a lesser period of time. When absent from the excretions, arsenic can no longer be found in the blood, though it may occur in the liver, bone marrow and kidney. Though from 50 to 65 per cent of the arsenic injected can be regained from the urine, nothing is known of the remaining 25 per cent or more.

The rarity of agranulocytosis due to arsphenamine, in view of the extensive use of this drug, suggests that in a certain few cases the bone marrow is idiosyncratic to this drug.

Bismuth—Agranulocytosis following bismuth therapy has been reported by Silduff and McMann, Mouquin and Fleury, Sézary and Boucher, Aubertin, Blankstein and Lehmann. It is reported associated with anemia or thrombopenia or both and of mild or severe grade. It is of interest to note that a good deal of bismuth is used in cases of ulcers of the stomach, gastro-enteritis, ulcerative colitis and diarrhea, and yet agranulocytosis has not been reported in this group of cases. Certainly syphilis has not been demonstrated to exert any rôle in disposing a patient to this complication.

Mercury and Silver—Some authors have suggested that mercury and silver are possible causes.

Gold—Extensively used in the therapy of chronic rheumatism, pulmonary tuberculosis, lupus erythematosus, leprosy and sarcoid, gold salts are responsible not only for cutaneous, mucous, digestive, renal and hepatic lesions but also for thrombopenic purpura, aplastic anemia and agranulocytosis. Since 1919 there have been about 30 cases of these blood dyscrasias reported, of which 2 (in women) were instances of agranulocytosis. Ameuille and Brailon, Jacob and Douady, Achard, Coste and Cahen, Emile-Weil, Jacquelin and Allanic, Emile-Weil and Bousser, Ramondi and San Giovanni, Bonafe and Mollard, Farjon, Laignel-Lavastine, Reye, Fourcade and Jauneau, Stigl, and Nardi have contributed to this subject. About 80 per cent of these cases occurred in women and all in France, perhaps because of the popularity there of gold as a medicament. Each of the gold products sanocrysin (sodium gold sodium thiosulfate), aurocantan, triphal, lopion (sodium gold thio-urea benzoate), solganol B (aurothioglucose), aurophos (the sodium gold double salt of an amino-arylphosphinic acid), colloidal gold and others have been responsible. The accidents occur at variable times during the course of injections, sometimes as early as the third or fourth injection. The symptoms are similar to those seen in "arsphenamine agranulocytosis", especially marked is fever with minimal or maximal hemorrhagic manifestations involving the skin and viscera.

Benzene—A close similarity exists between agranulocytosis and benzene poisoning, so much so as to suggest that the benzene ring

(contained in arsphenamine and in di-nitrophenol) is an etiologic agent Orzechowski, Barker, Flandin, Aubertin, Poummer, Baldrige and Hansmann and Kracke have each produced pure agranulocytosis using benzene experimentally. They discovered that the smaller the doses of benzene employed, the more selective the affinity for myeloid tissue. The picture of the destruction of white blood cells as seen in the bone marrow in agranulocytosis is closely identical to that seen in benzene poisoning. The experiments of Selling, who injected benzene into rabbits, are enlightening. Not only are the parenchymal cells of the hematopoietic tissue destroyed but the white cells of the peripheral blood. The myeloid suffers more than the lymphoid tissue, and the polymorphonuclears of the peripheral blood are more greatly affected than the lymphocytes. Though the erythroblastic tissue is destroyed, the erythrocytes of the peripheral blood are little affected. If benzene is given in adequate amounts the myeloid and lymphoid tissues can be made completely aplastic within three or four days, regenerative changes then begin, and after from fifteen days to three weeks these organs are completely regenerated. As regeneration progresses the blood picture approaches normal. In the bone marrow the process of regeneration begins with the formation of small circumscribed cell groups and islands composed of large lymphocytes, granulocytes and erythroblasts. These islands increase in size and number and then fuse with each other, and the reticulum becomes filled with parenchymal elements. In the early stages the young cells predominate with myeloblasts and myelocytes, and in the later stages they become rare. The small lymphocytes and polyblasts are most resistant to benzene and are found after the other cells disappear.

The pathologic picture is characterized by petechial and ecchymotic hemorrhages throughout all the viscera, the spleen becomes atrophic with the destruction of the lymphocytic elements, and the parenchymatous organs and bone marrow become fatty. With profound anemia, leukopenia and thrombopenia the bleeding time becomes increased, the blood clot fails to retract and the hemoglobin content is reduced. There is a decided lack of response to infection, symptomatically and experimentally (Winternitz and Hirschfelder, Camp and Baumgartner, and Kline and Winternitz). The studies of Horwitz and Dinker, Simonds and Jones and Hektoen have shown that there is a marked reduction in the thrombin-producing capacity, fibrinogen, lysins, precipitins and opsonins in the blood.

In many respects thorium, radium and x-rays have an effect that is very similar to that of benzene.

Di-Nitrophenol—Unlike benzene, arsphenamine, bismuth and gold, di-nitrophenol does not produce any blood dyscrasia other than agranulocytosis. In 1933 Tamter, Stockton and Cutting first called attention

to the use of this drug for the purpose of stimulating metabolism in the patient with hyperthyroidism and effecting loss of weight in the obese person. Its principal toxic effects are diaphoresis, hyperthermia, urticaria, angioneurotic edema, jaundice and nephritis, and tachycardia. a fall in blood pressure, acidosis and stupor also have been noted. It has produced agranulocytosis in the cases reported by Hoffman, Butt and Hickey, Silver, Davidson and Shapiro, and Bohn. Again, idiosyncrasy to this drug was blamed.

Aminopyrine—After Kracke suggested in 1931 the possibility that aminopyrine or its derivatives³ are important in the etiology of agranulocytosis, numerous reports (Watkins, Madison and Squier, Deversy Holten, Nielsen and Tiansbøl, Jorgensen, Costen, Barfred, Zimniger, Seemann, Andersen, Randall, Hoffman, Butt and Hickey, Laien, Rawls, Zinberg, Katzenstein and Wice, Benjamin and Biederman, Groen and Gelderman, Corelli, Fitz-Hugh, Knudson, Moltke, Johnson, Fisher, Sturgis, Plum and others) have appeared in which the

TABLE 4—Gatheicoal Survey of Prescription Ingredients on the Basis of Ten Thousand Prescriptions^{*}

	Hallberg Snow Survey of 1907	Charters Survey of 1927	U S Pharmacopoeia and National Formulary Survey of 1931-1932
Aminopyrine (U S P)	28	363	1358
Pyramidon	28	340	945

* Published by the American Pharmaceutical Association, 1933

drug is accused. Patented in Germany in 1896, aminopyrine has been used for the past twenty-five years in America and very extensively during the past fifteen years. According to the Gatheicoal Survey of Prescription Ingredients aminopyrine was prescribed by physicians almost sixteen times more frequently in 1926 than in 1907 and seventy times more frequently in 1931 and 1932 than in 1907. About 153 cases (Kracke and Parker) have been reported in which there was a history of its use. In only a very few of this number, however, had the condition been proved to be due to the pyrazolon drug. The importance of aminopyrine as a causative agent is illustrated by several facts. Experimentally, Madison and Squier produced agranulocytosis in a single rabbit given allonal (allylisopropyl barbituric acid with aminopyrine), Miller observed a decrease or almost complete absence of granulocytes in the bone marrow following its use, and Climenko has shown that after administration to the rabbit of aminopyrine, antipyrine,

³ According to Watkins the barbiturates and amytal have in rare instances been responsible for agranulocytosis. Neostibosan (an antimony compound) has also been blamed.

phenylhydrazine hydrochloride, catechol (pyrocatechin) and ortho-quinone the normal leukocyte response to nucleic acid is not evoked. Clinically, Madison and Squier, Plum, Benjamin and Biederman, Sturgis and others have reported cases in which the patients recovered from "aminopyrine agranulocytosis" and when small doses of aminopyrine were again administered the Schultz syndrome presented itself, some of the patients showing cutaneous reactions to the drug. When these patients ceased taking the drug an attack failed to recur. That aminopyrine can produce allergic phenomena is shown by the swelling of the eyelids, cheeks and lips, the blotches on the forehead and the backs of the hands, the malaise (Unger), the scrotal urticaria, the genital edema (Crohn) and the exacerbation of fixed eruptions, for which it may be responsible. The patch, intracutaneous and scratch tests give positive results for aminopyrine in certain rare cases (Meredith). These observations show that some persons possess an idiosyncrasy to aminopyrine. Also, some authors have expressed the belief that the striking incidence of agranulocytosis since 1922, its predominance in physicians, physician's families, housewives, medical students, nurses, hospital orderlies, druggists and dentists and its occurrence at the time of catamenia are to be explained by the use of aminopyrine and allied drugs. By virtue of these data it has been urged that the use of aminopyrine should be restricted or that when the drug is employed there should be careful observation of the peripheral blood picture.

That aminopyrine is only an occasional etiologic factor is supported by abundant experimental and clinical evidence. The attempt to produce agranulocytosis in the laboratory animal by the administration of aminopyrine has, for the most part, been a complete failure. Schilling, Kracke, Fitz-Hugh and Comroe, Maikson, Kunde and her associates and others have arrived at this conclusion. In an effort to investigate the effect of aminopyrine on the white blood cell and differential counts I undertook a study of 120 animals (guinea-pigs, rabbits and monkeys) over a period of eight months. Aminopyrine was given orally to one group in doses ranging from a fraction of a grain to 3 drachms (5.3 Gm.), daily, and blood cell and differential counts were taken daily. The symptoms were stupor, diarrhea and loss of weight. Many of the animals died of pneumonia, and some from aminopyrine poisoning, but at no time was agranulocytosis observed. This work was repeated with subcutaneous, intraperitoneal and intravenous injections, but no change in the blood picture occurred. With another group an attempt was made to sensitize some of the animals that were treated, and these were given a one month rest period, after which the medication was again given, still without effect. Incidentally, huge doses of the drug were injected into a pregnant guinea-pig for one and one-half months, but the new-born guinea-pig and the mother both had normal blood

pictures The next problem contended with was Do severe anemia, damage to the bone marrow, starvation, chronic infection and toxemia, or cachexia, constitute the soil on which agranulocytosis develops when aminopyrine is administered? The animals in the first group in this series were bled daily until the red blood cell count fell to between 1,500,000 and 2,000,000, and the red cells in the smear showed anisocytosis, poikilocytosis, and polychromasia Despite this preparation the white blood cell count failed to fall as a result of prolonged administration of the drug Into the animals in the second group small quantities of benzene in olive oil were injected subcutaneously until severe neutropenia and anemia were produced In most of the animals the white blood cell count fell to below 1,500 To one half of the animals in this group aminopyrine was given orally, and these animals showed, peculiarly enough, a more rapid rise in the white blood cell and polymorphonuclear differential counts than did the animals which were not given the drug After the animals in the third group were starved for five days the drug was administered but without avail In the animals in the fourth group broth cultures of *B subtilis*, *Str viridans* and *supestifer* were injected subcutaneously, and one month later, with the infection still persisting, they were given heavy doses of aminopyrine orally for three weeks The white blood cell counts were elevated during the period of aminopyrine treatment, and the differential counts showed a predominance of polymorphonuclears Thus, administration of aminopyrine to guinea-pigs, rabbits and monkeys, both with and without preliminary anemia or injury of the bone marrow, failed to produce any appreciable granulocytopenia

Many clinicians see no cause for alarm Schilling, Dennig and Zontscheff observed an aggregate group of 280 persons to whom aminopyrine was given daily for a period of a year, but no pernicious leukopenia was observed Gordon has noted that the various psychopathic institutions in the country where this drug is used constantly do not report the disease, nor has Archibald Hoyne observed this complication in the several contagious disease hospitals of Chicago where aminopyrine has been employed since 1929 as a more or less specific preparation for the therapy of measles Custer has added that at the Philadelphia General Hospital, having a yearly admission of about 30,000 patients, the approximate consumption of aminopyrine during 1934 was 120,000 tablets, no agranulocytosis was noted in the 1,926 autopsies performed during that year Aminopyrine is extensively used and in prodigious quantities in the United States, yet the idiosyncrasy to it is exceedingly rare

Some investigators have given aminopyrine to patients suffering from acute agranulocytosis and have observed no untoward results Jackson gave the drug to 3 patients, 2 of whom recovered and 1 died Limarzi

and Murphy administered a total of 370 grams (24 Gm) of aminopyrine to a patient during her fourth attack of acute agranulocytosis, and she survived. Marcus, similarly, gave doses of 2.5 Gm of aminopyrine to a subject and continued the administration for twenty-one days throughout the course of illness with no ill effects. At the March 1935 meeting of the German Congress of Internal Medicine, Naegeli, Dennig, Veil, Kissling, Neeigaard and Schlittenhelm said that they saw no reason for discrediting the drug.

In the light of present evidence aminopyrine is to be respected, like arsphenamine and bismuth, as a useful medicament but also as one to which a rare patient may be sensitive.

SUMMARY

Agranulocytosis is a disease of multiple etiology. Its aspect is being altered daily, and the tests of persistent clinical observation and experimentation are essential to mold it into acceptable form. At present all that is known is that agranulocytosis is a relatively new disease, which is universally distributed but especially throughout the United States, Germany, Denmark, England, France and Italy, that it attacks chiefly women between the ages of 40 and 60, that its essential process is one of degeneration of the myeloid cells in the bone marrow, causing a failure to replace the dying neutrophils of the peripheral blood stream, resulting in complete absence of the neutrophils and inviting septicemia, which often proves fatal several days after the insult to the bone marrow, that the "primary" form may be a result of infection, may be related to aplastic anemia or leukemia, may be a developmental defect or may be caused by vitamin deficiency or anaphylactic or endocrine factors, and that the "secondary" form develops from sepsis or in the rare person whose bone marrow is idiosyncratic to arsphenamine, bismuth, gold, di-nitrophenol, aminopyrine, barbitol and amytal medication. Greater intimacy with this disease will undoubtedly reveal fewer and fewer cases of idiopathic or primary origin.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths etc
—In Georgetown University, Washington, D C, Vincent J Dardinski, associate professor of pathology and parasitology, has been appointed professor of anatomy
N S Conant has been appointed associate professor of bacteriology at Duke University

Paul Kimmelstien has been appointed associate in pathology in the University of Pittsburgh

In the College of Medical Evangelists, Loma Linda, Calif, Albert Brown has been appointed instructor in pathology

John I Fanz, professor of pathology, bacteriology and hygiene in Temple University, Philadelphia, died Aug 26, 1935 He was 44 years old

Konrad E Birkhaug, from 1925 to 1932 associate professor of bacteriology at the School of Medicine and Dentistry of the University of Rochester and for the past three years a member of the Institut Pasteur at Paris, now occupies the chair of experimental medicine at Michelsen Institute for Scientific Investigations, Bergen, Norway

Frederick Robert Zeit, professor of pathology in the medical school of Northwestern University, Chicago, has died at the age of 71 years

Society News—The Georgia Association of Pathologists was organized Oct 29, 1935, with seventeen charter members The headquarters of the association will be in Atlanta The officers are Everett L Bishop, president, R R Kracke, vice-president, and T F Sellers, secretary-treasurer Meetings will be held monthly

The Pacific Northwest Society of Pathologists has been organized with the following officers F R Menne, president, O H Nickson, vice-president, T D Robertson, secretary-treasurer

Wisconsin Law on Blood Tests—This law follows the New York State law on blood tests and provides that in cases of disputed paternity the court may order "blood tests to determine whether or not the defendant can be excluded as being the father of the child" These tests may be made by "duly qualified physicians" and by "other duly qualified persons"

Grants in Aid—The next meeting of the Committee on Grants-in-Aid of the National Research Council will be held in March 1936 Applications for grants must be on file with the secretary, Clarence J West, 2101 Constitution Avenue, Washington, D C, not later than February 15 next

The next meeting of the Committee on Scientific Research of the American Medical Association for the consideration of applications for grants will be held early in February 1936 Applications should be addressed to the committee at 535 North Dearborn Street, Chicago

Obituaries

CHARLES NORRIS

1867-1935

Charles Norris was born in Hoboken, N J, on Dec 4, 1867. He died in New York on Sept 11, 1935. He attended Cutler's school in New York, obtained his collegiate degree from the Sheffield Scientific School of Yale University in 1888 and graduated from the College of Physicians and Surgeons, New York, in 1892. A two year internship at the Roosevelt Hospital followed. Here he came under the influence of Francis Delafield, one of the earlier American masters of pathology and an outstanding clinician of his time.

Following two years at Gottingen and Vienna, Dr Norris entered the department of pathology of the College of Physicians and Surgeons of Columbia University. Here, under the direction of T Mitchell Prudden, he carried out investigations in pathology, bacteriology and toxicology. In 1899 he became instructor in pathology at Cornell, but in 1902 he returned to the teaching staff of Columbia.

In 1904 Dr Norris gave up his full time academic career to become director of laboratories at the Bellevue Hospital. Here his talent for the organization and direction of scientific work, which was to become valuable to his community and to forensic medicine at large in his future career, had an opportunity for development.

The consolidation of its constituent parts into the greater city of New York led to intolerable conditions in the coroner's office. To the faulty functioning inherent in this office everywhere were added abuses and graft because a number of elective coroners, parts of whose jurisdictions had entered into the metropolitan consolidation, held authority in the greater city. In 1915 the state legislature abolished the office of coroner in the city of New York and replaced the divided authority of a number of coroners by that of a single office of chief medical examiner. This change was to become effective on Jan 1, 1918. In the interval, as the result of a comprehensive civil service examination conducted by a special committee of the outstanding pathologists of the city of New York, Dr Norris was appointed the first chief medical examiner. This office he held until his death.

It was as chief medical examiner that Dr Norris did his most important work, and outstanding work it was in a hitherto neglected field of medicine in the United States. The medical examiner system had been in use in Boston since 1877, but in New York the problem

was a much more difficult one because of the larger field to be covered by a single office and of the greater volume of work. To this task of organization Dr. Norris at once bent his unusual ability along this line. The task was an especially difficult one, not only because an entirely new type of governmental agency had to be developed but because many employees of the old coroner system, whose morals and morale had been contaminated by work under that system, had to be taken over. This factor impeded the work of the medical examiner for many years. How well the task of organization was accomplished becomes evident to any one who investigates the records, the record system and the work routine of the office.

To excellent previous training in pathology and its allied sciences and to ability in organization Dr. Norris added other attributes that were important for the success of the new venture. The large amount of actual physical work that he did in the performance of necropsies was possible only for one of his large frame and powerful physique. His tact and sense of humor, both attributes of value at times in a democracy, soon made it possible for him to convince the politicians that the office of chief medical examiner was not just another political office run by just another politician but an agency that tried to do important public work in a scientific manner. His imposing figure and his professional bearing made him an excellent expert witness, and his learning and experience, his unfailing good humor and the quizzical twinkle of his piercing eyes set under huge, shaggy brows put to rout the verbal pyrotechnics of bombastic cross-examining attorneys. The work of the chief medical examiner of the city of New York was soon accepted at its full value by police and prosecuting officials, courts, compensation boards and insurance companies.

Dr. Norris was a kindly man. Being a kindly man he could be deeply hurt by unjust criticism. And being a kindly man he was deeply appreciative of the good opinion of his colleagues. Early in 1935 the New York Academy of Medicine awarded him its gold medal for scientific achievement and outstanding public service. In reply to a note congratulating him on this honor, I received a letter, written in Dr. Norris' own characteristic angular chirography, brimming with enthusiasm and encouragement and sincere appreciation.

Dr. Norris had been assistant professor of pathology in the medical school of Cornell University and professor of pathologic anatomy in the medical department of Fordham University. In 1933 he was made professor of forensic medicine in New York University and Bellevue Hospital Medical College.

The place of Dr. Norris as chief medical examiner will be hard to fill. But his successor will find a firm foundation of work already

accomplished on which to build further. If and when the rest of the country replaces the antiquated coroner system by the more modern and scientific agency of the medical examiner, the medical examiner's office of the city of New York will become the most fitting monument to the ability and memory of Charles Norris.

OSCAR T. SCHULTZ

Abstracts from Current Literature

Pathologic Anatomy

EFFECT OF PERITONEAL RESORPTION ON THE LIVER E G LAIRD, *Virchows Arch f path Anat* **291** 440, 1933

A variety of substances in solution were injected intraperitoneally into mice and rats, and their effects on the liver studied histologically, to test out the thesis that substances resorbed from the peritoneal cavity by the blood stream are carried directly to the liver. The chemicals used were potassium iodide, sodium iodide, iodoform, arsenic, yellow phosphorus, manganese chloride, and ortho and meta toluyldiamine. The dosages used were such as proved fatal in from twenty-four to forty-eight hours. At short intervals after injection the animals were killed and subjected to histologic examination. Within fifteen minutes to one hour after injection marked fatty change of the hepatic parenchyma was evident. This progressed to vacuolar degeneration of the hepatic cells. Intravenous injection of the same chemicals had a much less marked effect on the liver. Ligation of the portal vein preceding intraperitoneal injection prevented or delayed the toxic action on the liver. The greater part of the injected potassium iodide was excreted in from three to six hours and of iodoform in from twenty-four to forty-five hours. The chemicals appeared in the bile within a short period after injection. It is suggested that intraperitoneal administration may be a valuable therapeutic procedure when a rapid and direct action on the liver is desired.

O T SCHULTZ

XANTHOMA OF THE HEART K WOLFF, *Virchows Arch f path Anat* **293** 472, 1934

A man aged 62 years had had an acute rheumatic infection fifteen years before his death. Seven years before death signs of chronic cardiac embarrassment appeared. A month and a half previous to death the symptoms of acute endocarditis developed. Necropsy revealed a chronic stenosing aortic endocarditis, recent ulcerative endocarditis of the aortic and mitral valves with perforation into the right auricle, and carcinoma of the esophagus. Especial attention is directed to the presence of groups of foam cells filled with cholesterol in the fatty tissue at the hilus of the kidney, in a mesenteric lymph node and in the heart, in which the right ventricular wall was most markedly involved. In the heart the foam cells occurred in areas of fibrosis in which there was more recent cellular proliferation. Wolff asks whether the xanthomatous condition and the valvular disease were separate and distinct processes or whether and in what manner the two processes were related to each other. His analysis leads him to conclude that the endocarditis and the lipid deposition were distinct processes, but that they had a sequential relation to each other. The acute valvular disease activated the older inflammatory areas in the myocardium. In the presence of hypercholesteremia, which was not established by antemortem chemical examination and which is tentatively ascribed to the esophageal carcinoma, cholesterol was deposited in the activated granulation tissue. He suggests that inflammation in the presence of disturbed cholesterol metabolism may be an essential factor in Hand's (Schuller-Christian) disease.

O T SCHULTZ

A CASE OF CONGENITAL ENCEPHALITIS H BRANDT, *Virchows Arch f path Anat* **293** 487, 1934

In 1866 Virchow applied the name "congenital encephalitis" to a condition in new-born infants and young children characterized by areas of fatty degeneration

and sclerosis in the brain The cerebral condition may be associated with palsies, idiocy or Little's disease The cerebral lesion has in general been ascribed to trauma sustained during birth In the case reported by Brandt, that of an infant who lived two days, the presence in the brain of areas of necrosis that had progressed to calcification indicated that the factor causing the cerebral lesions had acted during intra-uterine life This factor he suggests may have been a circulatory disturbance, possibly transient in its action

O T SCHULTZ

HYPERPLASIA OF BRUNNER'S GLANDS F FEYRTER, *Virchows Arch f path Anat* **293** 508, 1934

As a result of the careful examination of the duodenums of 2,800 subjects Feyrter recognizes and describes three forms of benign hyperplasia of Brunner's glands diffuse and nodose hyperplasia, circumscribed nodular hyperplasia with single or multiple nodules, and adenoma The first two forms were observed in 0.85 per cent of the cases studied Circumscribed benign adenoma is more rare and was observed in 0.12 per cent There was a significant but not absolute correlation between chronic renal damage and hyperplasia of Brunner's glands This is ascribed to reactive hyperplasia following injury to glandular epithelium by excreted toxic substances Adenomas are not the result of tissue misplacement Previous investigators had noted hyperplasia of Brunner's glands at the margin of duodenal ulcers, Wolff observed a similar process in the scars of healed ulcers

O T SCHULTZ

ASBESTOSIS BODIES E BINTKLER, *Virchows Arch f path Anat* **293** 527, 1934
P J BEGER, *ibid*, p 530

This is a rather hair-splitting and controversial reply and rebuttal called forth by a previous article of Beger Bemtler maintains that the silicic acid liberated from inspired asbestos particles evokes a connective tissue proliferation the localization of which is different from that of the usual form of silicosis The asbestosis bodies are the result of a protective reaction, they are pathognomonic of asbestosis but have no bearing on the clinical manifestations of the process Beger admits that the harmful action of asbestos is due to liberated silicic acid but insists that the process in asbestosis in itself is no different from that in other forms of silicosis The asbestosis bodies increase the liberation of silicic acid and hence are an essential factor in the more severe clinical course and the greater degree of tissue reaction in asbestosis as compared with silicosis When asbestosis bodies are not formed, asbestosis may run as benign a course as ordinary silicosis

O T SCHULTZ

PRIMARY TUBERCULOSIS OF THE ESOPHAGUS J INCZE, *Virchows Arch f path Anat* **293** 540, 1934

Tuberculosis of the esophagus may occur as the result of implantation, and then it may be primary, of involvement by direct continuity from adjacent tuberculous foci, or of hematogenous or lymphatic transport The condition described by Incze was noted in a 10 year old idiot who had swallowed a solution of lye twenty months before death Recovery was followed by stricture of the esophagus Death was preceded by symptoms of tuberculous meningitis Necropsy revealed a stricture of the esophagus at the lower level of the larynx and another at the level of the tracheal bifurcation Between these the esophagus was dilated and was the site of caseous tuberculosis Epithelium was absent in the tuberculous region There was generalized miliary tuberculosis with no foci older than the esophageal lesion The case is one of primary implantation tuberculosis in an esophagus scarred by the action of a caustic

O T SCHULTZ

ATHEROSCLEROSIS OF THE AORTA AS OBSERVED IN ONE THOUSAND NECROPSIES OF PERSONS OVER TWENTY YEARS OF AGE E DORMANN and E EMMINGER, Virchows Arch f path Anat **293** 545, 1934

In this investigation the factors taken into account were age by decades, sex, the degree of atherosclerosis of the various portions of the aorta (ascending portion, arch, descending portion and abdominal portion) and the degree of arteriosclerotic involvement of the mouths and of the peripheral portions of the coronary arteries. These data were analyzed separately for tuberculosis, carcinoma and syphilis, all other conditions being grouped together as controls. The incidence and severity of atherosclerosis was relatively slight in the ascending portion of the aorta, increased in the arch, decreased again in the descending portion, and again became more marked in the abdominal portion. Except for syphilis, the curves are much alike for the diseases studied and for the controls. In syphilis, involvement was marked as to both incidence and severity in the three thoracic portions of the aorta, it was somewhat less marked in the abdominal portion but above the level for tuberculosis and carcinoma and the controls. Severe atherosclerosis of the abdominal portion of the aorta was more frequent in women than in men and coronary arteriosclerosis was more frequent in men. These differences in the sexes became more evident with advancing years up to the age of 70. Severe atherosclerosis was less frequent in cancerous persons than in the controls, the percentages for the various decades being higher for women than for men. Coronary sclerosis was relatively less frequent in the syphilitic group than in the others. In tuberculous persons the incidence of abdominal atherosclerosis was relatively lower and that of coronary sclerosis relatively higher.

O T SCHULTZ

A MESENTERIC CYST WITH THE STRUCTURE OF AN ESOPHAGUS S FLODERUS, Virchows Arch f path Anat **293** 608, 1934

A mesenteric cyst removed from a girl aged 11 years had a length after fixation of 7 cm and a diameter of 2.5 cm. Its lumen was lined in greater part by multilayered squamous epithelium like that of the esophagus. The epithelium rested on a thick muscularis. In places the lining was like intestinal mucosa, with single-layered columnar epithelium and crypts of Lieberkuhn. Groups of mucous glands were embedded in the wall. Floderus postulates an origin from pluripotent tissue separated from the entoderm in early embryonic life.

O T SCHULTZ

HISTOLOGY OF THE PANCREAS IN NUTRITIONAL DISTURBANCES OF NURSINGS ST MAHRBURG, Virchows Arch f path Anat **293** 682, 1934

Histologic examination of the pancreases of thirty nurslings dead of nutritional disturbances revealed no characteristic or specific changes. The interacinar tissue was edematous and relatively prominent as the result of passive congestion.

O T SCHULTZ

GYNECOMASTIA AND CIRRHOSIS OF THE LIVER M BERGONZI, Virchows Arch f path Anat **293** 697, 1934

Reserving the term "gynecomastia" for diffuse non-neoplastic hypertrophy of the male mamma, Bergonzi notes that this condition is most often associated with cirrhosis of the liver, pulmonary tuberculosis, syphilis and tumor of the testis. From the Italian literature he has tabulated 101 examples of such association, many of the reports are chival. Of 453 cases of gynecomastia recorded in the world's literature 60 (13 per cent) showed the association with atrophic cirrhosis of the liver. The association was first fully discussed by Silvestrini in 1923, mammary hypertrophy in hepatic cirrhosis is known as Silvestrini's sign. Bergonzi has previously reported 2 cases and reviewed the subject in an Italian monograph. In the present article he reports the findings in an additional case and discusses

the pathogenesis of gynecomastia in cirrhosis. The patient was a man aged 54 years at the time of death. Symptoms of cirrhosis of the liver became evident two years before death. At about the same time loss of sexual power was noted, this progressed to complete impotence. The gross and microscopic observations may be summarized as follows: interlobular atrophic cirrhosis of the liver with hemosiderosis of the liver, spleen, pancreas, hypophysis, thyroid and testes, atrophy of the hypophysis with increased colloid content of the pars intermedia and basophilic infiltration of the posterior lobe, atrophy and tubular degeneration of the testes, slight hyperactivity of the thyroid, bilateral glandular mammary hypertrophy. The pathogenesis of gynecomastia in cirrhosis of the liver is ascribed by Bergonzi to endocrine dysfunction, especially of the testes and hypophysis. In support of this view of the endocrine feminizing effect of hepatic cirrhosis in the male he notes that the female type of pubic escutcheon is observed so frequently in cirrhosis as to be of diagnostic importance, and he himself has frequently noted atrophy and tubular degeneration of the testes in cirrhosis. That gynecomastia does not occur more often is probably due to an underlying constitutional factor which modifies the feminizing effect.

O T SCHULTZ

HYDATID MOLES IN CATTLE. A F FOLGER, *Acta path et microbiol Scandinav*, supp 1934, p 104

This disease in cattle is rare, only four cases have been reported previously. Folger discusses in detail five cases of his own, and concludes that in cattle so-called hydatid mole frequently develops as a hyperplasia of the connective tissue of the chorion with no involvement of the epithelial elements. The morphologic variations are summarized as follows: (1) a formation of villi with or without cysts, (2) polymorphous tissue-like nodules that develop possibly from certain intravascular proliferations, (3) a structure similar to the choriocarcinoma of human beings which apparently arises from hypertrophic villi in which cysts form. Folger calls attention to the associated occurrence in cattle of hydatid mole and teratoma.

Immunology

COMPLEMENT FIXATION AS RELATED TO RESISTANCE AND ALLERGY IN EXPERIMENTAL TUBERCULOSIS. A B BAKER, *Am Rev Tuberc* **31** 54, 1935

In experimental work with tuberculosis vaccinated rabbits can be differentiated from nonvaccinated ones by the complement-fixation test. The titer of the antibody in vaccinated animals does not measure the existing allergy but appears to parallel the resistance of the animals to experimental tuberculosis. Antibody which has been produced by vaccination and which cannot be detected by the complement-fixation test is not destroyed but has left the circulation, it can be exfoliated by various stimuli.

H J CORPER

THE DECOMPOSITION OF TUBERCULOPROTEIN, STARCH AND GELATIN BY DR GRINDING. C H BOISSEvain, *Am Rev Tuberc* **31** 542, 1935

Prolonged grinding of starch causes it to change first into erythrode\tr\in and then into achroodextrin. Prolonged grinding of gelatin changes it into a peptone-like compound that is very soluble in cold water and unable to form a gel even at icebo\ temperature. Defatted tubercle bacilli consist mainly of an insoluble compound of specific polysaccharide and tuberculoprotein. This compound can be broken up by prolonged grinding into water-soluble protein, peptone and polysaccharide. Treatment with 4 per cent sodium hydroxide solution or boiling with dilute acid has the same effect. Water-soluble protein is hydrolyzed into peptone in dilute solutions.

H J CORPER

A COMPARISON OF THE ANTIGENIC PROPERTIES OF DEFATTED TUBERCLE BACILLI AND THEIR DERIVED PROTEINS C H BOISSEVAIN, *Am Rev Tuberc* **31** 547, 1935

Water-soluble proteins obtained from ground tubercle bacilli, from hydrolyzed bacilli and from filtered culture medium have the same tuberculin activity. Doses of from 1 to 40 mg of defatted tubercle bacilli make guinea-pigs hypersensitive to 0.1 cc of a 0.01 per cent solution of tuberculo-protein. The same doses of the insoluble residue obtained by grinding make guinea-pigs hypersensitive to 0.1 cc of a 1 per cent solution. It was not possible to make guinea-pigs hypersensitive to a 1 per cent solution of tuberculo-protein by the injection of from 10 to 100 mg of any water-soluble or alkali-soluble protein or peptone derived from the tubercle bacillus

H J CORFEE

TETANUS TOXIN NOT TRANSPORTED IN PERIPHERAL NERVES J J ABEL et al, *Bull Johns Hopkins Hosp* **56** 84, 1935

We have presented many considerations and many facts in support of our belief that tetanus toxin and dyestuffs injected in an aqueous medium intraneurally, subcutaneously, intramuscularly or intravenously are not carried in the axis-cylinders, the lymphatic vessels or the tissue spaces of peripheral motor nerves to the reacting cells of the central nervous system. We have also cited the recent investigations of anatomists who have traced the outflow of lymph from nerve trunks and have shown that it, like the lymph of other structures of the body, is added finally to the venous blood and not to the cerebrospinal fluid. We furthermore called attention to a series of investigations that were carried out by Abel and Abel and Turner in the years 1910 to 1914 in which it was conclusively shown that alkaloids and dyestuffs cannot be distributed throughout the body by any peripheral mechanism such as the "tissue spaces"

FROM THE AUTHORS' SUMMARY

INTRADERMAL ANTISERUM TESTS L FOSHAY, *J Allergy* **6** 360, 1935

Intradermal tests with certain antisera, e g, antitularense, antibrucella, etc, may cause the typical reaction of sensitization in the allergic sense as well as—and this is a new observation—simple erythema with central edema and complete absence of urticaria in patients with the corresponding infection. The reaction is specific and unrelated to sensitization with the serum. The erythematous-edematous reaction may be useful as a diagnostic aid. Approximately 0.04 cc of a 1:10 dilution of the antiserum should be injected intradermally. The reaction usually appears within a few minutes.

THE OCCURRENCE OF ANTIFIBRINOLYTIC PROPERTIES IN THE BLOOD OF PATIENTS WITH ACUTE HEMOLYTIC STREPTOCOCCUS INFECTIONS W S TILLET, *J Clin Investigation* **14** 276, 1935

A specific antiserum directed against the fibrin-dissolving action of hemolytic streptococci was demonstrated in the blood of approximately 75 per cent of patients who recovered from acute streptococcal infections. The fibrin clot from the blood of patients who died of the infection was in no instance capable of inhibiting the bacterial fibrinolytic action. The antifibrinolytic response was demonstrable at the approximate time of recovery in some patients, and in others it was not detected until the second to fourth week of convalescence. Although there were exceptions, the specific response appeared during the course of erysipelas earlier than it did during that of any acute streptococcal infection of the upper respiratory tract. Antifibrinolytic resistance developed in twenty-eight patients. All of them recovered, and their convalescence was not interrupted by a reactivation of the streptococcal infection. Seventeen patients failed to acquire humoral antifibrinolytic properties. In seven of those included in this group the disease was self-limiting and convalescence uneventful. Of the remaining ten

seven died and three had prolonged illnesses due to exacerbations of active streptococcal infection. In a few cases of active rheumatic fever and acute nephritis which were studied antifibrinolytic resistance was usually present. The response did not differ in its development from that which occurred in cases of acute streptococcal infection without the visceral involvements of rheumatic fever or acute nephritis.

FROM THE AUTHOR'S SUMMARY

ANAPHYLAXIS WITH POLLEN C. BERNSTEIN JR., J. Exper. Med. **61** 149, 1935

Guinea-pigs given intracutaneous and subcutaneous injections of relatively small amounts of an extract of the pollen of burweed marsh-elder did not show anaphylactic response to intravenous shock doses of this material three weeks later. Animals sensitized with horse serum before or at the time of the injection of pollen extract could be shocked after an interval of three weeks with pollen extract alone. The possible role of this underlying sensitivity is discussed.

FROM THE AUTHOR'S SUMMARY

HYPERSENSITIVENESS IN DOGS AFTER EXPERIMENTAL PNEUMOCOCCUS LOBAR PNEUMONIA L. T. COGGESHALL, J. Exper. Med. **61** 235, 1935

The data derived from the investigations indicate that dogs do not acquire hypersensitivity to *Pneumococcus* as the result of experimental lobar pneumonia. This inference is based on the following findings. Fifteen dogs were given type I and type II pneumococcal lobar pneumonia and following recovery were tested for hypersensitiveness by means of intrabronchial and intracutaneous injections of the autolysate of the homologous type of *Pneumococcus*. Seven dogs had a pulmonary lesion discernible in the x-ray film at the site of the intrabronchial injection of the autolysate, three of these dogs were normal controls. No evidence of a positive skin reaction was found in any of the fifteen dogs, many of which received repeated infections and intradermal injections of the autolysate. Subsequent infections in the same animals were definitely milder than the initial infection. The infections following the intrapulmonary and cutaneous administration of the autolysate were practically of the same intensity as the initial infection. The temperatures, pulse rates, white blood counts and differential blood pictures showed no significant variations following intrapulmonary injection of the autolysate. Tests for the acquisition of humoral immune bodies following the injection of the autolysate and recovery from the experimental disease showed the presence of these substances in some dogs and their absence in others. Histologic study of the pulmonary lesions produced by the autolysate failed to reveal changes characteristic of an allergic reaction. However, the perivascular accumulations of large mononuclear cells observed in the lesions of the dogs which recovered suggest a locally accelerated reactivity of the fixed tissue cells to the products of *Pneumococcus*.

FROM THE AUTHOR'S SUMMARY

THE SEROLOGICAL DIFFERENTIATION OF HEMOLYTIC STREPTOCOCCI FROM PARTURIENT WOMEN R. C. LANCEFIELD and R. HARE, J. Exper. Med. **61** 335, 1935

The majority of the strains of hemolytic streptococci from puerperal uterine infections were identified serologically as members of the group A described by Lancefield. The majority of the strains isolated from the birth canals of women in whom the puerperium was afebrile were not members of group A. Two new serologic groups of hemolytic streptococci, F and G, are described.

FROM THE AUTHORS' CONCLUSION

LOCAL SKIN REACTIVITY TO *BACILLUS TUBERCULOSIS* G. SHWARTZMAN, J. Exper. Med. **61** 369, 1935

New toxic substances in certain tuberculin, old tuberculin and filtrates of *Bacillus typhosus* are described. These substances are capable of eliciting the

hemorrhagic necrosis characteristic of local skin reactivity provided heterologous bacterial filtrates of high potency are used for either the intradermal or the intravenous injection. The toxic substances apparently have no relationship to the tuberculin substances proper. The experiments with inactive preparations also demonstrate in rabbits a state of hypersensitiveness to tuberculin, old tuberculin and bacteria-free culture filtrates in the absence of tuberculous foci. The reactions are elicited provided the tissues are rendered vulnerable through contact with certain soluble bacterial factors capable of eliciting local skin reactivity to bacterial filtrates and provided the tubercle bacillus culture filtrate is injected intravenously into immunized rabbits.

FROM THE AUTHOR'S SUMMARY

GRADING OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES G SHWARTZMAN,
J Exper Med **61** 383, 1935

In this paper are described methods for grading local skin reactivity to bacterial filtrates. The grading is accomplished by titration of the skin-preparatory factors and by studies of the duration of the ensuing reactivity. It was found that the duration of reactivity depends on the mode of preparation of the filtrates and on the micro-organisms employed. Thus, it lasts for ninety-six hours with *Meningococcus* "agar washings" filtrates and for seventy-two hours with *Bacillus typhosus* "agar washings" filtrates, it disappears within forty-eight hours with *B typhosus* tryptic digest broth culture filtrate and *B typhosus* "agar washings" filtrates previously heated in the Arnold sterilizer for twenty minutes. Comparative titrations of the preparation employed demonstrate that the duration of reactivity is in direct relationship to the reacting potency. It is also shown that the skin-preparatory potency of the filtrates and the duration of the ensuing local reactivity are not modified by cellular anaphylactic sensitization (Arthus' phenomenon) to animal proteins. The exposure of tissues to the effect of certain soluble bacterial factors induces a high susceptibility to humoral toxic principles resulting from intravascular antigen-antibody interaction.

FROM THE AUTHOR'S SUMMARY

THE NEUTRALIZATION TEST IN POLIOMYELITIS J R PAUL and J D TRASK,
J Exper Med **61** 447, 1935

The difference between two human and two passage strains of the virus of poliomyelitis when tested by this method amounted to about 25 per cent, and there was less power in normal adult serums to neutralize human than passage strains of virus. The difference between the two human strains amounted to 15 per cent, and that between the two passage strains to 8 per cent, the last figure falling within the limits of the experimental error of the method. The extent to which these findings affect certain concepts with regard to the epidemiology of poliomyelitis based on neutralization experiments with passage strains cannot be determined from the data presented in this paper, except that they more or less confirm the view previously derived from such experiments that from 70 to 95 per cent of normal urban adults possess in their blood a substance which neutralizes the virus in a given amount. However, certain other indications appear when the present results are supplemented by those previously obtained (Paul and Trask *J Exper Med* **56** 319, 1932, **59** 513, 1933). Primarily, the authors have found no relation between the clinical acquisition of poliomyelitis and the presence of a substance in the serum which neutralizes a passage strain of the virus. With a passage strain the results seem rather to bear a closer relationship to age than to illness. With a human strain results have been obtained in which there is some evidence, shown only in the juvenile group, that acquisition of the clinical disease is accompanied by the appearance of antiviral properties in the blood.

FROM THE AUTHORS' SUMMARY

THE PRECIPITIN REACTION BETWEEN TYPE III PNEUMOCOCCUS POLYSACCHARIDE AND THE HOMOLOGOUS ANTIBODY M HEIDELBERGER and F E KENDALL, *J Exper Med* **61** 559 and 563, 1935

The precipitin reaction between the specific polysaccharide of *Pneumococcus* type III and the homologous antibody formed in the horse can be accounted for quantitatively by assuming the chemical combination of the components in a bimolecular reaction, followed by a series of competing bimolecular reactions which depend on the relative proportions of the components. These reactions lead to the formation of larger and larger aggregates until precipitation ultimately occurs. The mathematical formulation of this theory on the basis of the law of mass action (Guldberg and Waage) is described. The derived expressions are shown to be in accord with the experimental findings, and the constants used in these expressions are shown to have definite significance. In spite of the wide variation in the properties of individual serums these expressions permit complete description of the behavior of an unknown serum with S III without an unduly burdensome number of analyses. The quantitative theory presented has been found applicable to other instances of the precipitin reaction, as will be shown in subsequent papers.

FROM THE AUTHORS' SUMMARY

LOCAL DISPERSION OF INFECTIOUS AGENTS AS A FACTOR IN RESISTANCE F DURAN-REYNALS, *J Exper Med* **61** 617, 1935

Progressively decreasing quantities of bacteria of some twenty strains were utilized in experiments to determine the effect of dispensing the organisms in the rabbit's skin through the agency of an extract of testicle or an invasive staphylococcus. The same experiment was made with six strains of filtrable viruses. When the organisms introduced were above a certain number or quantity (the minimal effective concentration) the bacterial lesions were enhanced by spreading, on the other hand, when the number was less than this the lesions were partially or totally suppressed. The virulence and the minimal effective concentration were observed to be in inverse relationship. The lesions due to the filtrable viruses were enhanced by spreading even when the quantity of virus approached the minimal infective dose. This happened whether the virus caused severe or slight lesions. When *Pneumococcus* of the highly virulent type I was injected with the spreading factor into normal rabbits it yielded enhanced lesions even at practically the minimal infective dose, but when the resistance of the animal was raised with specific antiserum the lesions were totally suppressed owing to the dispersion of the bacteria. When such an experiment was repeated with a filtrable virus, that of vaccinia, no suppression took place as a result of the dispersion of the infective agent.

FROM THE AUTHOR'S SUMMARY

THE FIXATION AND PROTECTION OF VIRUSES BY THE CELLS OF SUSCEPTIBLE ANIMALS P ROUS, P D McMASTER and S S HUDACK, *J Exper Med* **61** 657, 1935

Methods were developed for a study of the relations existing between viruses and living cells. It was found that the virus of vaccinia and the virus causing the infectious fibroma of rabbits (Shope) rapidly become fixed on tissue cells freed as individuals and submitted to the viruses in suspension. This happens whether the cells are alive or have been killed with heat or ultraviolet rays. The virus does not come away during agitation of the cells with Tyrode solution and repeated washings with large amounts of it. The exposure of cells carrying virus to neutralizing antiserum fails to affect the virus significantly if the cells are alive, whereas if they are dead the activity of the virus is nullified. Cells freed as individuals from tissue cultures of the virus of vaccinia and the Shope tumor carry these viruses in abundance through repeated washings and if living protect them from the influence of neutralizing serum, whereas killed cells exert no such protection. The findings appear to throw light on the way in which viruses gain a

foothold in the host, and they suggest reasons for the persistence of some viruses in animals that have recovered and for the unsatisfactory results of serum therapy instituted during the course of virus diseases. The virus causing the Shope fibroma has been successfully maintained in cultures of the growth. It is closely associated with the cells, almost none being present in the culture fluid. Certain of its other attributes have been determined. The virus of vaccinia greatly damages the cells of cultures of rabbit embryo in which it is under propagation.

FROM THE AUTHORS' SUMMARY

THE TYPE I MENINGOCOCCUS SPECIFIC SUBSTANCE H. W. SCHERP and G. RAKE, *J. Exper. Med.* **61** 753, 1935

This substance has been isolated and purified. It appears to be a sodium salt of a polysaccharide acid.

FROM THE AUTHORS' SUMMARY

THE FORMATION OF AGGLUTININS WITHIN LYMPH NODES P. D. McMASTER and S. S. HUDACK, *J. Exper. Med.* **61** 783, 1935

Agglutinins are formed within the draining lymph nodes of mice following intradermal injections of killed cultures of micro-organisms.

FROM THE AUTHORS' SUMMARY

CONCENTRATION OF ANTIBODY IN IMMUNE SERUM JOHN H. HANKS, *J. Immunol.* **28** 95, 1935

A gelatinized solution of antigen furnishes a practical means of determining the antibody content of both weak and strong antisera. The titer of as little as 0.03 cc. of serum can be determined by allowing an optimal amount of antigen to diffuse into increasing dilutions of the antisera and reading the dilution in the last tube in which a ring is visible on the upper layer of the gelatinized antigen.

ELIZABETH MCBROOM

THE MECHANISM OF THE TUBERCULIN REACTION J. H. HANKS, *J. Immunol.* **28** 105, 1935

If a foreign protein (egg-white, horse serum) is injected into a tuberculous lesion of a guinea-pig or into an apparently normal testicle adjacent to an infected testicle there develops a specific cutaneous hypersensitivity like that characteristic of the tuberculin reaction. A possible approach to the mechanism of the tuberculin reaction is suggested by this variation in the type of response to a foreign protein of an animal receiving an injection of the protein at the site of a tuberculous lesion.

ELIZABETH MCBROOM

HYPERSENSITIVENESS AND IMMUNITY TO FOREIGN PROTEINS F. H. TEALE, *J. Immunol.* **28** 161, 1935

Hypersensitivity is due to that state of the tissues in which they cannot deal with specific antigen even when it is completely saturated with the homologous antibody. In immunity the tissues are increasingly capable of dealing with the antigen as the degree of immunity increases, the circulating antibody is of little moment. The greater amount of circulating antibody is not the main difference in the states of increasing immunity to the protein in question. In order to produce passive hypersensitivity definite amounts of antibody are necessary, the amounts vary with the antigen against which the antibody is specific, again showing that the titer of the antibody *in vitro* is no exact measure of its effectiveness in the body. There is no special antibody causing hypersensitivity, the antibody seems to be related to the ordinary lytic or precipitating one. These results again

show the preeminence of the state of the tissues in regard to immunity, of their capability of dealing effectively with the antigen, and the negligible part played by the circulating antibody as such

FROM THE AUTHOR'S CONCLUSIONS

THE PHOTODYNAMIC EFFECT OF METHYLENE BLUE ON TETANUS TOXIN K M LIPPERT, *J Immunol* 28 193, 1935

The toxic properties in a filtered broth culture of *Clostridium tetani* are rapidly diminished by the photodynamic action of methylthionine chloride (methylene blue) in certain concentrations. The inactivation of the tetanus toxin in the presence of dilute solutions of methylthionine chloride is more marked after exposure of the mixture to light for one hour than after shorter intervals of time. The antigenic property could not be demonstrated in tetanus toxin after partial destruction of its toxicity by exposure to light in the presence of dilute solutions of methylthionine chloride. An optimum concentration of this drug for the inactivation of the toxin by light is demonstrated. In the concentration used it does not inhibit the effect of the toxin in vivo

FROM THE AUTHOR'S SUMMARY

A QUANTITATIVE STUDY OF SERUM PRECIPITIN IN ANAPHYLAXIS IN THE RABBIT C JACKSON, *J Immunol* 28 225, 1935

A high titer of precipitin for ovalbumin is not a guarantee that an animal can be fatally shocked by an injection of ovalbumin. The amount of ovalbumin necessary to produce shock does not bear a constant relationship to the titer of the precipitin. The highest percentage of deaths from shock occurred among female rabbits, the majority of these being pregnant at the time of the experiment

ELIZABETH McBROOM

ARE ANTIBODY ACTIONS DUE TO SEPARATE ANTIBODIES? F H TEALL, *J Immunol* 28 241, 1935

Different manifestations of antibody activity are not due to the activity of the same immune substance acting differently under different physical conditions, etc., but are due to the activity of different antibodies. This can be shown by the difference in their development, by the observation that in some cases the agglutinin can be adsorbed, leaving the protective power of the antiserum intact, and by the fact that heat at 65 C destroys the protective power and leaves the agglutinin uninjured. Attention has also been drawn to the fact that a serum may have a high titer of agglutinin and yet have no protective power and vice versa

THE RÔLE OF PRECIPITIN IN THE REMOVAL OF INTRAVENOUSLY INJECTED ANTIGEN J T CULBERTSON, *J Immunol* 28 279, 1935

Rabbits immunized against horse serum can dispose of horse serum injected intravenously more readily than can normal rabbits. Immune rabbits with circulating precipitin are somewhat better able to dispose of horse serum injected intravenously than are immune rabbits which have been permitted to rest until circulating precipitin has disappeared. Crystallized egg albumin injected intravenously in small amounts into specifically immunized rabbits cannot be detected in the circulation after its injection. The precipitin content of the serum of these animals is diminished following the injection of the antigen into the blood stream. It appears likely that the antibody both in the circulation and in the fixed tissues unites with the antigen introduced into the circulation. The quantity of antibody immediately available for such union is evidently much less in the fixed tissues than in the blood, the amount in the tissues being usually not over 25 to 50 per cent of that in the blood. When an amount of antigen significantly greater (by from 25 to 50 per cent) than that calculated to neutralize the circulating antibody is introduced all of the immediately available antibody is removed from the circulation

and from the fixed tissues and antigen can be detected in the blood. The amount of circulating precipitin against crystallized egg albumin is not significantly altered by the injection of numerous nonspecific substances into the circulation.

FROM THE AUTHOR'S CONCLUSIONS

AN IMMUNOLOGICAL STUDY OF THE EFFECTS OF INTENSE SOUND VIBRATIONS ON EGG ALBUMIN. E. W. FLOSDORF and L. A. CHAMBERS, *J. Immunol.* **28** 297, 1935.

The coagulum produced by sonic irradiation of a solution of egg albumin has a much decreased antigenic activity and an altered specificity. The altered specificity of the coagulum is similar to the specificity of albumin denatured in other ways. The soluble fraction remaining after sonic irradiation is unaltered immunologically. Prolonged irradiation increases the amount of coagulum produced and then redisperses it into an opalescent sol of the same altered serologic specificity. Very prolonged irradiation decreases the amounts of both serologically native and altered proteins. Excellent agreement with respect both to antigenic activity and specificity was observed in the results with the Arthus phenomenon, the intradermal tests and the precipitin reactions. In some cases the intradermal test was somewhat more delicate than the precipitin reaction.

FROM THE AUTHORS' SUMMARY

THE FLARING UP OF INJECTION SITES IN ALLERGIC GUINEA PIGS. L. DIENES and F. A. SIMON, *J. Immunol.* **28** 321, 1935.

In a guinea-pig that has been given an intracutaneous injection of human serum or turtle egg a flare-up at the site of injection can often be observed after a few days. This occurs at the time when skin sensitiveness begins to develop, usually on the fourth to sixth days. Both the flare at the original site and the skin test at this time are purely of the delayed type, and anaphylactic shock cannot be produced in the guinea-pig. In the reacting flare about the original site of inoculation the tissues show infiltration with mononuclear cells, which is characteristic also of the delayed type of skin reaction. It is pointed out that the developing allergy probably plays a role in the development of specific tissue reactions in the lesions of certain infectious diseases.

FROM THE AUTHORS' SUMMARY

THE INDIVIDUAL AS A FACTOR IN ANTIDIPHTHERIA IMMUNITY. J. M. NEILL, J. Y. SUGG and L. V. RICHARDSON, *J. Immunol.* **28** 363 and 385, 1935.

The investigation is essentially an analysis of the antitoxin titers of the serums of a large number of guinea-pigs immunized by different methods and bled at six month intervals between the first and the third year following the last injection. The data indicate that the individual animal is a more important factor than the method of immunization in determining the titer of antitoxin at times remote from the last injection. Examples are given of the wide differences in individual guinea-pigs in respect to their loss of antitoxin. Some of the animals had such a marked capacity to maintain their immunity that their titers appeared approximately "level" over long periods, the time at which this "level" was reached and the height at which it was maintained appeared to be as much a characteristic of the individual guinea-pig as of the method of immunization.

FROM THE AUTHORS' SUMMARY

THE SPONTANEOUS OCCURRENCE OF BRUCELLA AGGLUTININS IN DOGS. W. H. FELDMAN, F. C. MANN and C. OLSON JR., *J. Infect. Dis.* **56** 55, 1935.

By agglutination tests agglutinins of *Brucella* were found to be present in the serum of 52, or 10.4 per cent, of 500 dogs obtained as adults from rural environments. Serums from 15 of the reactors failed to give agglutination of the antigens.

in dilutions greater than 1:12, whereas serums of 33, or 66 per cent, gave positive reactions in dilutions of 1:25 or greater. Serums of only 5 of the animals showed agglutination in dilutions of 1:100 or greater. Attempts were made to isolate *Brucella abortus* by inoculation of guinea-pigs with emulsions of tissues from 14 of the reacting animals, the results were negative. Dissemination of the infection from dogs with agglutinins against *Brucella* by cohabitation with normal dogs failed. None of the dogs with agglutinins against *Brucella* exhibited symptoms indicative of specific infection, and in none whose bodies were examined at necropsy was there pathologic evidence of disease due to *Br. abortus*. A study was made of the possible cross-agglutinability of *Br. abortus* and *Bacillus bronchisepticus*. Dogs and rabbits were utilized. Cross-agglutination of significant degree did not occur.

FROM THE AUTHORS' SUMMARY

ANTIGENIC CHARACTERISTICS IN MAN OF CERTAIN PRODUCTS OF THE PNEUMOCOCCUS. L. D. FELTON, W. D. SUTLIFF and B. F. STEELE, *J. Infect. Dis.* **56** 101, 1935

Certain fractions of the pneumococcus, treated under various conditions, produce in the human being a protective antibody response comparable in degree to that obtained with vaccine. However, the response to the various antigenic fractions differs from that to type I and type II vaccines to this extent. While the response to type I vaccine is largely homologous and that to type II vaccine only moderately heterologous, the response to the various fractions, though in different degrees is much more heterologous.

Tumors

THE SCHUBERT-DANNMEYER TEST FOR CANCER. E. R. HOLIDAY and F. C. SMITH, *Am. J. Cancer* **23** 339, 1935

A study of the diagnostic test for cancer devised by Dannmeyer, Schubert and Treplin is described. Forty-seven cases of cancer and forty-six control cases have been investigated. Dannmeyer's claim to a diagnostic accuracy of 95 per cent cannot be confirmed, though a certain degree of specificity seems indicated. The test in its present form is insufficiently reliable and too complicated for regular clinical use.

FROM THE AUTHORS' SUMMARY

THE ACTION OF 1:2:5:6-DIBENZANTHRACENE ON *OBELIA GENICULATA*. S. P. REIMANN and F. S. HAMMETT, *Am. J. Cancer* **23** 343, 1935

Observations made on the development of *Obelia geniculata* in cultures in sea water containing 1:2:5:6-dibenzanthracene to approximate saturation and in control cultures in plain sea water and in sea water plus an equivalent amount of solvent for the test compound, show that the substance increases the proliferation of this organism.

FROM THE AUTHORS' SUMMARY

RETROPERITONEAL XANTHOGRANULOMA. C. OBERLING, *Am. J. Cancer* **23** 477, 1935

Xanthogranuloma consists of inflammatory tissue infiltrated more or less extensively by foam cells containing cholesterol ester, cholesterol and neutral fats. The abundant proliferation of histiocytic or fibrocytic elements in some areas suggests a true tumor process, but xanthogranuloma is to be distinguished from xanthoma and xanthosarcoma by its histologic polymorphism which, on the whole, is that of an inflammation and not that of a tumor. Xanthogranuloma forms the anatomic substratum of Hand-Rowland (Schuller-Christian) disease, but it may also be observed other than in this condition, in various regions of the body. A characteristic localization appears to be that in the retroperitoneal space, where the xanthogranuloma may extend widely and simulate a tumor of considerable size. Three such cases are described together with the cases previously published in the literature.

FROM THE AUTHOR'S SUMMARY

CONDITIONS INDUCED BY OESTROGENIC COMPOUNDS IN THE COAGULATING GLAND AND PROSTATE OF THE MOUSE H BURROWS, *Am J Cancer* **23** 490, 1935

The prolonged administration of estrin to mice causes hyperplasia and metaplasia in the prostate. The prostate undergoes similar changes in children before birth, presumably under the influence of estrin derived from the placenta. Both in mice and in new-born children the prostatic changes thus induced are completely reversible, restitution to the normal state following a cessation of the supply of estrin. The changes found in benign enlargement of the human prostate in advanced life resemble in character and situation those observed in mice under the influence of estrin and in new-born babies. The question is discussed whether the benign enlargement of the prostate in elderly men may be due to the action of estrogenic hormones and may be a reversible condition.

FROM THE AUTHOR'S CONCLUSIONS

EXTRAMEDULLARY PLASMA-CELL TUMORS OF THE UPPER AIR PASSAGES W L MATTICK and A A THIBAudeau, *Am J Cancer* **23** 513, 1935

A case of plasma cell tumors apparently neoplastic occurring simultaneously in the nasopharynx and hypopharynx, is described. In the nineteen instances previously reported by other observers the plasma-cell tumor has been variously interpreted as granulomatous, inflammatory, benign or malignant. The details of these cases are presented in tabular form. The occurrence of polypoid growth of single or multiple nature in the upper air passages with a history of long duration should arouse suspicion of a plasma cell tumor. The use of the x-rays aids in diagnosis. It is believed that the tumors reported are unusual and dissimilar from most of the tumors previously reported in that they are definitely neoplastic.

FROM THE AUTHORS' SUMMARY

GRANULOSA-CELL TUMORS OF THE OVARY H C THORNTON, *Am J Cancer* **23** 522, 1935

A case of granulosa cell tumors in a 63 year old woman six years past the menopause is described. There was a history of vaginal bleeding beginning about one year after the cessation of menstrual bleeding and continuing intermittently for four years, then becoming almost continuous for about a year. Examination revealed a large uterus and a movable pelvic mass. At operation a right ovarian tumor 6 cm in diameter, the right tube and the body of the uterus were removed. The ovarian tumor proved to be a typical granulosa cell tumor showing folliculoid, cylindromatous and diffuse areas. The histologic structure was predominantly cylindromatous. Grossly, the tumor was practically solid, showing only a few small cysts. The uterus was greatly hypertrophied, and the endometrium showed marked glandular-cystic hyperplasia. There was also an adenomyoma of the uterus. The urine collected over a period of thirty-one hours, beginning twenty-four hours after the operation, was shown to contain folliculin. The diagnosis of granulosa cell tumor in this case rests on the morphologic characteristics of the tumor, the evidence of follicular hormone activity in a woman six years past the menopause presented by the large uterus, hyperplastic endometrium and history of uterine bleeding, and the demonstration of folliculin in the urine.

FROM THE AUTHOR'S SUMMARY

EFFECT OF GOITER-PRODUCING DIETS ON THE GROWTH OF CARCINOMA, SARCOMA AND MELANOMA IN ANIMALS K SUGIURA and S R BENEDICT, *Am J Cancer* **23** 541, 1935

In all groups of animals fed on a goitrogenous diet or the same diet containing added iodine (80, 8,000 or 800,000 γ per hundred grams of food) the rate of tumor growth was slow. This was probably due to the impairment in the health of the animals produced by the faulty diet irrespective of the iodine content. The per-

centage of tumor takes and tumor regressions was found to vary significantly with variation of the iodine content of the diets. The lowest number of takes and the greatest number of tumor regressions occurred in those animals receiving a normal amount of iodine, while animals on either high iodine or low iodine diets showed a high percentage of takes and a low percentage of regressions. Prolonged feeding of the high iodine diets (the animals were fed a normal, well balanced diet containing excessive amounts of potassium iodide) had no therapeutic effect on the growth of Flexner-Jobling rat carcinoma, mouse sarcoma 180 and Pacey mouse melanoma.

FROM THE AUTHORS' SUMMARY

THYROID ADENOMA IN EXPERIMENTAL ANIMALS C. A. HELLWIG, *Am J Cancer* **23** 550, 1935

For the first time in North America thyroid adenomas have been observed in white rats receiving a calcium-rich goitrogenous diet. Thyroid nodules originate not from fetal rests, but from differentiated thyroid epithelium. They are due to the same stimulus which causes diffuse hyperplasia. Thyroid nodules, after reaching a certain size, show all the characteristics of true adenomas. They represent an intermediate stage between hyperplasia and malignant tumor.

FROM THE AUTHOR'S SUMMARY

ADAMANTINOMAS OF THE HYPOPHYSEAL STALK AND SPHENOID BONE H. ZEITLIN, *Am J Cancer* **23** 729, 1935

Tumors of the hypophyseal stalk arise from a definite location but may show histologic differences. They may present a typical arrangement of the epithelial elements and then are usually classified as adamantinoma, some may present atypical enamel formation, others display evidence of malignancy. Cystic degeneration is usually a predominant histologic feature. Clinically the foregoing types cannot be differentiated, but a general diagnosis of an extrahypophyseal tumor can be made.

FROM THE AUTHOR'S CONCLUSION

ENTDIFFERENTIATION IN BRONCHIOGENIC CARCINOMA P. C. SAMSON, *Am J Cancer* **23** 741, 1935

In a review of microscopic entdifferenciation in thirty-four cases of primary bronchogenic carcinoma it has been shown that it is possible to arrange these cases in a series with progressive gradations from the most highly differentiated types to those showing extreme entdifferenciation. In the arrangement of the series three fundamental neoplastic cell types have been used: the columnar cell (adenocarcinoma), the squamous cell and the undifferentiated cell carcinoma. When the columnar cell and squamous cell carcinomas are arranged in separate series as to progressive entdifferenciation they approach a common type in which it is no longer possible to distinguish the parent cell. Below this level of differentiation the histologic picture closely resembles that of sarcoma. Cell type in bronchogenic carcinoma depends on the kind and degree of differentiation attained, and not on the level of origin in the respiratory tract. For each of the twenty cases not previously reported a brief description of cell type and extent of metastasis has been given.

FROM THE AUTHOR'S SUMMARY

THE RELATION OF CELL TYPE TO METASTASIS IN BRONCHOGENIC CARCINOMA P. C. SAMSON, *Am J Cancer* **23** 754, 1935

A survey was made of 100 cases of bronchogenic carcinoma in which there were complete autopsies with adequate microscopic control. These were classified as to cell type and 51 per cent were found to exemplify columnar cell (adenocarcinoma), 30 per cent squamous cell and 19 per cent undifferentiated cell carcinoma. A comparison of the reported metastases in the three groups was made by the

method of coefficients of association, and it was found that each group presented certain general characteristics. Adenocarcinoma showed a strong tendency to involve the central nervous system, adrenals, kidneys, both lungs and to some extent the liver. This is best explained on the basis of predominant hematogenous metastasis, with involvement also of the thoracic and abdominal lymph nodes. Squamous cell carcinoma showed a marked tendency to local extension rather than to widespread metastasis, involving the pericardium and bronchial lymph nodes to some extent. In 24 per cent of the cases of squamous cell carcinoma no pulmonary metastases were found. Because of the tendency to local extension this group would appear to offer the best prognosis for radical removal. Judged from the type of organ involved, the small cell carcinoma showed little tendency to hematogenous metastasis. The neoplasms in this group showed extensive lymphogenous metastases, however, associated with involvement of the pancreas, liver and spleen. Because of the differences in the type and extent of growth and of metastases, knowledge of the cell type and of the degree of differentiation can be of aid to the clinician in determining the most advisable course of treatment. Such knowledge should obviously be used in conjunction with the physical examination, history and roentgenographic study. FROM THE AUTHOR'S SUMMARY

A GLIOMA IN A DOG AND A PINEALOMA IN A SILVER FOX (*VULPES FULVUS*)
C F SCHLOTTIAUER and J W KERNOHAN, *Am J Cancer* **24** 350, 1935

GROWTH-PROMOTING AND GROWTH-INHIBITING SUBSTANCES FROM NORMAL ORGANS J MAISIN and Y POURBAIX, *Am J Cancer* **24** 357, 1935

The tissues of the liver, pancreas and intestinal mucosa promote growth of cancer in tarred mice. The brains, thymus, bone marrow, dried gastric mucosa and dried lymph nodes exert an inhibitive action on the development of tar cancer. The same organ—brain, for example—may contain both growth-inhibiting and growth-promoting factors. The growth-promoting substances are for the most part soluble in water at pH 7 and pH 8.4 and are relatively insoluble in ether, being extracted by the latter solvent in only small quantities, with the exception of cholesterol. The growth-inhibiting substances are soluble in ether or are removed by it. They are relatively insoluble in acetone, the soluble portion being precipitated by calcium. The antianemic factor added to the diet in pure form has no influence on growth of tar cancer.

FROM THE AUTHORS' CONCLUSIONS

THE HORMONAL ORIGIN OF UTERINE FIBROIDS AN HYPOTHESIS J T WITHERSPOON, *Am J Cancer* **24** 402, 1935

Pathologic and clinical evidence is offered to support a hypothesis that there exists a cause and effect relationship between the unopposed and persistent action of the estrogenic principle produced by multiple follicular cysts of the ovaries on the uterine endometrium and myometrium and the production of immediate endometrial hyperplasia and (provided the stimulation is sufficiently prolonged) more latent uterine fibroids. Since this estrogenic principle affects the genital tract as a whole and also controls the development of the mammary glands it appears that this same hormonal stimulation is the factor initiating endometrioma and fibro-adenoma of the breast.

FROM THE AUTHOR'S CONCLUSIONS

HYPERPROTEINEMIA IN MULTIPLE MYELOMA ALVIN G FOORD, *Ann Int Med* **8** 1071, 1935

In four cases of multiple myeloma, profound autohemagglutination of the red cells occurred in dry and wet films, and difficulty was encountered in counting erythrocytes in two cases because of granule formation in the pipet. Hyperproteinemia was proved to be present in three cases and was apparently present in the fourth, and seemed to be the cause of these abnormal phenomena. Renal

insufficiency apparently resulted from plugging of the glomerular capillaries by inspissated protein and possibly by the clumping of erythrocytes or changes in osmotic pressure due to the increased protein. Intravascular clumping was demonstrated during life by ophthalmoscopic examination of the retinal veins in one case when pressure sufficient to slow down the circulation was applied externally on the eyeball. Prolonged uncontrollable bleeding and clotting time and increased calcium content of the serum with little change in the platelet count occurred in two cases. The findings of marked rouleaux formation in the smears in two of the cases led to procedures finally indicating the diagnosis of multiple myeloma. Multiple myeloma with resultant renal abnormalities should be suspected in cases of atypical nephritis.

FROM AUTHOR'S SUMMARY (FRANK R. MENNE)

LIPOMA OF INTESTINE J. D. KIRSCHBAUM, *Ann Surg* **101** 726, 1935

In 5,754 autopsies lipoma of the intestine was found 9 times. In 7 of these cases the lipoma measured from 1 to 3 cm in diameter, in 1 case it became detached and caused obstruction of the ileum, in the remaining case the lipoma caused intussusception of the lower part of the ileum into the cecum.

ORIGIN AND NATURE OF PIGMENTED NEVI (SCHWANNOMAS) S. W. BECKER, *Arch Dermat & Syph* **30** 779, 1934

Examination of several hundred pigmented nevi by modern histologic methods showed that they consisted of one or two types or a combination of both types. The first type is strictly cellular and results from the multiplication of the clear cells in the epidermis with or without penetration into the dermis, the second type is of nerve origin and is deeper, with structures which often simulate those of tactile corpuscles. The use of the term "schwannoma," as suggested by Masson, is not illogical, although more work must be done before the origin of clear cells and of nevi from the sheath of Schwann can be proved. The term "nevus" and "nevoid" are ambiguous and could be replaced by the term "prenatal" without losing any of their significance.

FROM THE AUTHOR'S SUMMARY

LEUKEMIC SINUS RETICULOSIS (MONOCYTIC LEUKEMIA) R. B. HAINING, S. KIMBALL and O. W. JANES, *Arch Int Med* **55** 574, 1935

The venous sinuses of the spleen and of the bone marrow and the lymph sinuses of the spleen and of the lymph glands probably are lined by differentiated reticular cells. These cells are similar to the Kupffer cells of the liver. Like the Kupffer cells, they are entirely different from the endothelial cells lining the ordinary blood and lymph channels. The term "reticulo-endotheliosis" applied to monocytic leukemia does not elucidate the fact that the endothelial cells lining the ordinary blood and lymph vessels have nothing to do with the process. Neither does it attempt to distinguish between true reticulum and sinus reticulum (reticulo-endothelium). Monocytic leukemia is probably a sinus reticulosis, whereas myeloid and lymphoid leukemia are probably true reticuloses. There are good grounds for the conception that leukemia is a malignant neoplastic process. Malignant processes and neoplasia are closely associated in our minds with infiltration and with tumor formation. Specific localization with tumor formation is apparently infrequent in leukemic sinus reticulosis (monocytic leukemia). A case is recorded of acute monocytic leukemia in a young woman with localized mucosal and submucosal reticulosis in the rectal wall which assumed the proportions of a tumor. The tumor produced complete intestinal obstruction, clinically, the case exactly simulated carcinoma of the rectum with intestinal obstruction.

FROM THE AUTHORS' SUMMARY

Society Transactions

LOS ANGELES PATHOLOGICAL SOCIETY

Regular Meeting, March 12, 1935

A G FOORD, *President, in the Chair*

J W BUDD, *Secretary*

PATHOLOGY OF VAGINITIS DUE TO TRICHOMONAS JOHN F KESSEI and JAMES A GAFFORD JR

Seventy cases of vaginitis in which the classic clinical symptoms of vaginitis due to *Trichomonas* were exhibited and the flagellate *Trichomonas vaginalis* was recovered were observed in this study. Clinically, the walls of the vagina were usually found to be injected and tender, presenting in some instances hyperemia and often minute points of hemorrhage. In the more advanced cases granular areas were prominent. Sections of biopsy material taken from these hemorrhagic and granular areas in forty cases were studied with special reference to the tissue changes and the presence or absence of the trichomonads in the lesions.

The surface in some areas was covered with coagulated material in which trichomonads, white cells and red cells were commonly found. The surface epithelium was usually intact but in certain areas showed erosion, with flagellates between the cells. Areas of hemorrhage were also occasionally observed to break through the surface epithelium.

In the mucosa were areas of infiltration containing collections of lymphocytes, a few polymorphonuclears and an occasional plasma cell. These areas showed increased vascularity and hemorrhage and extended in some instances through the surface epithelium. At times definite necrosis occurred within these areas. Usually it was not possible to find trichomonads in the areas of early infiltration, while in the definitely necrotic regions flagellates were present, and in one case in which the epithelium was entirely eroded they were found in the deeper non-necrotic tissues.

These observations indicate that the pathologic changes are not brought about by simple invasion of the flagellates from the mucosal surface, but that some other mechanism, either toxic or bacterial, is responsible for the primary change of tissue, or that the flagellate is conveyed by the blood stream to the areas of infiltration and ultimate necrosis.

Bacteriologic studies of the vaginal exudate from thirty of the patients with vaginitis and of the secretion from twelve normal persons showed streptococci of the alpha type and also diphtheroids to be present in the secretions of the majority of the normal persons, as well as in the exudates from patients with trichomonad vaginitis. Aciduric bacteria, presumably of the Doderlein type, were found less frequently in the patients with vaginitis than in the normal persons. Gram-negative rods of the colon bacillus type were found in 25 per cent of the cases.

Transplanting exudate containing both *T. vaginalis* and bacteria from patients with vaginitis to three normal volunteers has resulted in the occurrence of typical symptoms and in recovering *Trichomonas* from all of the volunteers. Implanting cultures of *T. vaginalis* and the bacteria with which they grew also produced typical symptoms in two of the three volunteers. In one, the bacteria alone produced mild symptoms of vaginitis.

Attempts to establish *Trichomonas* of the human intestine in the human vagina have met with negative results. Attempts to establish *T. vaginalis* in the intestinal tracts of kittens have been negative, although *Trichomonas intestinalis* of man may be transmitted to kittens. These observations support the theory that the trichomonads of the vagina and the trichomonads of the intestine are distinct species.

TETANY IN A SIX WEEK OLD INFANT C M HYLAND

A 6 week old boy with a history of convulsions that started when he was approximately 5 weeks of age was admitted Jan 21, and died Jan 25, 1935. Birth had been normal with a gestation period of eight months. The weight at birth was 8 pounds and 2 ounces (3,685.4 Gm). The infant was breast fed for the first month and progressed normally for about five weeks, until the convulsions started. The convulsions were described by the parents as follows. The child would shake violently and draw up all extremities, and in the intervals would sleep deeply. The convulsions occurred only in the evening hours. The family history was not relative. Four other children were alive and well.

The patient appeared physically normal except that the head had a peculiar feel interpreted as that of craniotabes. The tuberculin test was negative. The blood showed hemoglobin, 66 per cent, red blood cells, 3,010,000, white blood cells, 10,600, polymorphonuclears, 27 per cent, lymphocytes, 55 per cent, monocytes, 14 per cent, eosinophils, 2 per cent, promyelocytes, 2 per cent. The spinal fluid had a trace of globulin but was normal otherwise. The blood calcium was 6.8 mg per hundred cubic centimeters of serum and the blood phosphorus 7.6 mg per hundred cubic centimeters of whole blood. X-ray pictures of the head and wrists revealed nothing of importance.

While the boy was in the hospital, many convulsions were noted and he was given 10 Gm of calcium chloride daily, with considerable symptomatic relief. The convulsions became much less severe but did not entirely cease.

The convulsions were not typical of tetany as carpopedal spasm was not observed. However, the condition was regarded as tetany associated with rickets regardless of the negative roentgen report. While there was improvement following calcium therapy the course continued to be downhill, and the child died four days after admission.

The only gross pathologic observations at autopsy were acute follicular colitis of a minor degree, hyperplasia of the mesenteric lymph glands and acute hyperplasia of the spleen. The brain was normal. The craniotabes thought to be present was not verified by autopsy or by X-ray picture.

Microscopic examination showed no irregularity of the zone of ossification in the costochondral and costospinal regions. The primary and secondary zones of ossification were normal. The organs of the neck were removed *en masse* and multiple serial sections were made of the thyroid and of the thyroid portion of the trachea. In all, 1,500 sections were examined for the presence of parathyroid bodies, and two such bodies were recognized, a larger one embedded in the thyroid gland, measuring 250 microns in greatest diameter, and a smaller one embedded in the tissue surrounding the trachea, measuring 50 microns in greatest diameter. The former appeared normal, but the smaller one showed marked vacuolation of the cells.

The features supporting the view that the spasmodic attacks and death were of the nature of tetany due to parathyroid aplasia are (1) the extremely small amount of parathyroid tissue compared with the normal, (2) the absence of any other recognized causes of tetany, including rickets, which was ruled out by microscopic examination of the bones, (3) the abnormally low blood calcium and high blood phosphorus, and (4) the symptomatic relief following the administration of calcium.

Tetany due to aplasia or absence of parathyroid tissue is unusual, and it is for this reason that the case is presented.

PATHOGENESIS AND PATHOLOGIC ASPECTS OF OTOGENOUS INTRACRANIAL LESIONS
CYRIL B. COURVILLE and J. M. NIELSEN

Many problems concerned with the intracranial extension of infection in the middle ear and mastoid are as yet unsolved. With some of these problems in mind, we reviewed the records of 10,000 consecutive autopsies. There were 51 instances of otogenous dural infection (2 of chronically thickened dura, 19 of

extradural abscess, 6 of pachymeningitis, 17 of subdural abscess and 7 of dural fistula), 69 instances of thrombosis of venous channels (35 of the lateral sinus, 9 of the cavernous sinus, 8 of the superior longitudinal sinus, 4 of the superior petrosal sinus, 3 of the inferior petrosal sinus and 10 of cerebral veins), 128 instances of septic meningitis (89 primary and 39 secondary), and 35 instances of encephalic abscess (17 of the temporal lobe, 12 of the cerebellum, 3 of the parietal lobe and 3 of the frontal lobe). The 283 lesions were found in 167 cases. The incidence and description of the various lesions we have reported in other contributions (Courville, C B, and Nielsen, J M *Arch Otolaryng* **19** 451, 1934, *Acta oto-laryng* **21** 19, 1934, *Arch Surg* **30** 930, 1935).

One interesting aspect of the complications of otitis media is the seasonal variation in their incidence. For example, in the fiscal year 1929-1930, in 859 autopsies, complications of otitis were found in 63 (7.3 per cent), 32 (51 per cent) being extracranial and 31 (49 per cent) intracranial. Reports of only 4 cases of thrombosis of venous channels were found. In 1932-1933, in 1,414 autopsies, complications of otitis media were found in 109 (7.8 per cent), 67 (61 per cent) being extracranial and 42 (39 per cent) intracranial. There were reports of 15 cases of thrombosis of venous channels in this year, almost four times as many instances in less than twice the number of autopsies.

One indication of an extension of infection to the cerebrum is jacksonian seizures usually of the opposite arm and side of the face, at times followed by weakness or actual paralysis of the affected members. This syndrome is particularly frequent in children (Courville, C B, and Nielsen, J M *Am J Dis Children* **49** 1, 1935). In older persons these symptoms frequently follow thrombosis of the lateral sinus. The cerebral lesion is located in the region of the central sulcus and may be a temporary local ischemia and edema, actual softening or an abscess. When these symptoms precede those indicative of meningitis, it is likely that the meningeal infection began in this region. Since in a number of cases these symptoms follow thrombosis of the lateral sinus, it is likely that they are due to retrograde extension along the inferior communicating vein (anastomotic vein of Labbe) to the central region. It is furthermore likely that retrograde extension along the communicating system of venous channels accounts for the location of abscesses in the frontal, parietal and occipital lobes, rather than metastasis via the general circulation, even though these channels are not grossly occluded.

The possibility of the development of a subdural abscess secondary to otitis media is coming to be more widely recognized. In this series of autopsies 17 cases of otogenous subdural abscess were found. These may be due (1) to direct spread of infection by contiguity through bone and dura into either the middle or the posterior fossa, (2) to retrograde extension along venous channels or (3) to spontaneous or traumatic rupture of an encephalic abscess in the subdural space. Only when subdural collections of pus become large and when they are uncomplicated with other lesions do they constitute a diagnostic and therapeutic problem.

More study needs to be given to the pathogenesis of abscess of the brain. Three anatomic types have been described: (1) the small, heavily encapsulated abscess, (2) the larger circumscribed abscess with thin capsule and (3) the diffuse abscess (suppurative encephalitis). Any of these three types may occur in the cerebellar white substance as well, and, in addition, small or large interfoliar abscesses may occur. One type of abscess is largely preventable—the postoperative or so-called traumatic abscess. In this case the exploring needle has made its approach through an infected field and carried the infection into the brain. Such a lesion is to be thought of when symptoms of an abscess arise shortly after an exploration.

MIXED TUMOR OF FUNDUS UTERI ALVIN G. FOORD

Twenty-three cases of mixed tumors of the body of the uterus, including instances of the tumor diagnosed as rhabdomyosarcoma, have been reported in the literature. Most of these have shown embryonic striated muscle, and about a third

have shown glands, cartilage or rarely other structures such as bone, fat or nerve tissue. The following case was clinically and pathologically similar to most of the cases reported in that the tumor occurred in a woman past the menopause, was a polypoid growth, recurred soon after removal, did not respond to radiation, and was formed in the greater part of embryonic striated muscle.

A woman, aged 59, the mother of four children, four years past the menopause consulted her physician for uterine bleeding of about three weeks' duration. Just before admission to the hospital she suffered labor-like pains in the pelvis. The uterus was moderately diffusely enlarged. From a markedly dilated external os a large soft polypoid growth with a necrotic tip protruded. The uterus above the portio vaginalis cervicis was removed the following day. The pelvic lymph nodes and the tubes and ovaries were free from change. The uterus was symmetrical and measured 11 by 9 by 6 cm. The endometrial cavity was dilated and filled by a tumor mass 10 by 9 by 7 cm, which arose, by a narrow pedicle about 1.5 cm across, from the midportion of the posterior wall of the fundus. Cut surfaces showed no gross extension into the thinned musculature of the fundus. The surfaces were soft, glistening white and homogeneous except for a few areas of hemorrhage or necrosis, chiefly in the distal end.

Microscopically, large areas of the tumor were composed of edematous myxomatous tissue and others of interlacing spindle cells with little or no intercellular matrix. Most of these cells contained hyperchromatic nuclei, and mitoses were frequent. In sections stained with hematoxylin and eosin, and especially in those prepared with iron-hematoxylin stains, cross-striations of some of the cells could be seen. In addition, many huge cells were found with single or multiple nuclei, in many of these, interlacing small fibers showing cross-striations were prominent, in some, only a highly eosinophilic granular cytoplasm was found. Strewn throughout part of the tumor were small nests of hyaline cartilage and gland groups or single bizarre glands composed of cells differing moderately in size and shape and showing hyperchromatic nuclei. These had no connection with the endometrium. Invasion of the uterine muscle was found in the base of the tumor.

A recurrence of the tumor occurred within three months, and when the patient was seen five months after operation, a large mass was palpable above the pubic brim. Treatment with x-rays of high voltage in large doses was given for six weeks with no response. The patient died seven months after first being seen.

At autopsy a huge tumor, 21 by 17 by 16 cm, filled the entire pelvis, and a continuation extended nearly to the umbilicus. There were metastases in the iliac and periaortic and peripancreatic lymph nodes and a few implants on the visceral peritoneum, but there was no involvement of the lungs or liver. Four small nodules were found in the thyroid. Microscopically, the metastases were entirely rhabdomyosarcomatous. No cartilage or glands were seen. Spindle cells with and without striations, similar to those in the original growth, were abundant. They apparently represented embryonic striped muscle cells too immature to show striations.

PHILADELPHIA PATHOLOGICAL SOCIETY

HERBERT L. RATCLIFFE, *Secretary*

May 9, 1935

MORTON McCUTCHEON, *President, in the Chair*

AYERZA'S DISEASE D. R. COMAN

A white woman, aged 35 years, entered the Hospital of the University of Pennsylvania on April 20, 1935. In 1927, eight days after appendectomy and right oophorectomy, she contracted pneumonia and never thereafter entirely regained health. She complained of pain in the back, which had persisted for some time, and

of a sore throat. In December 1934 she had had a cold, and since that illness she had noted progressive somnolence, dyspnea and headaches. The blood pressure was 130 systolic and 90 diastolic, the erythrocyte count was 8,120,000 with hemoglobin 124 per cent, and the leukocyte count was 7,500. The heart was enlarged, and a rough systolic murmur was heard over the pulmonary area.

The patient rapidly became worse, dyspnea increasing and the blood pressure falling. On May 3 paralysis of both legs with loss of pain and touch sensation developed, and death occurred within a short time.

At autopsy the body was that of an obese white woman of plethoric habitus, weighing 65 Kg and measuring 160 cm in length.

The heart weighed 390 Gm. The right auricle was greatly dilated and engorged with blood. The walls of the right and left ventricles were of equal thickness, 1.3 cm, and the apex was formed by the right ventricle. The aortic valve was of the bicuspid type, and the cusps contained calcific plaques.

The aorta was apparently smaller than normal, as were its main branches. It presented yellow intimal thickenings, but no definite gross signs of syphilis. Just distal to the origin of the left subclavian artery, the aorta presented a constriction which barely permitted the little finger to enter its lumen. The pulmonary artery presented several calcareous plaques just above the valvular cusps.

The lungs were partially collapsed. They had emphysematous borders and a subcrepitant doughy consistency. Cut surfaces exposed very prominent vascular structures. The smaller and medium-sized ramifications of the pulmonary arteries stood elevated above the rest of the lung surface as gaping tubes. Their walls were somewhat thickened. The larger vessels were dilated.

At the site of the right ovary there was a small cyst attached by a twisted pedicle. The right ovarian vein was thrombosed near its lower extremity.

The spinal cord showed a thrombosis of the posterior spinal artery.

The gross anatomic diagnosis was Ayerza's disease.

Microscopic examination of the lungs revealed striking changes in the blood vessels. Many of the smaller arteries and arterioles showed complete obliteration by fibrosis and hyalinization. Medium-sized branches showed vacuolation of the medial coat, in which muscle fibers and fat were increased. The intima was thickened and in many instances showed infiltration by small round cells. Stains for elastic tissue showed the internal elastic lamina thickened and split, with a general increase in the elastic elements of the vascular wall.

In comparing this case with those that have been described it is found to present the typical features of primary pulmonary sclerosis with hypertrophy of the right ventricle. Clinically, cases of this type are frequently mistaken for polycythæmia vera, as this one was. Interesting here are the additional observations of coarctation of the aorta and thrombosis of the posterior spinal artery.

ENDOMETRIOSIS OF THE APPENDIX A. R. CAMERO and F. MOGAVERO

A white woman, aged 23, married, was admitted to the Misericordia Hospital in September 1934 with a chief complaint of pain in the right lower quadrant of the abdomen. She had been well until about a month before admission, when she had her first attack of abdominal pain on the right side. A diagnosis of acute appendicitis was made, and operation was advised, but she refused surgical intervention, and the attack gradually subsided. The pain recurred, however, at irregular intervals. Because of the continuance of the annoying sensation in the appendical area, she finally submitted to operation.

Her menstrual history was normal except that she had missed one period a few weeks before admission to the hospital. Physical examination gave essentially negative results except for slight tenderness in the right lower quadrant of the abdomen. The urine and the blood counts were normal.

At operation (by Dr. George P. Muller) the appendix was found low in the pelvis. Its tip was covered with granulation tissue which bled easily. The uterus was palpated, it was soft and enlarged to the size of a six weeks' pregnancy. The

appendix measured 7 cm in length and 9 mm in diameter. The distal fourth was kinked and covered with delicate vascular adhesions. The bulbous distal fourth was firm and on cross-section showed an eccentric thickening of the appendical wall with a markedly contracted lumen, displaced to one side. The serosa covering the rest of the appendix was smooth.

Microscopically, a fairly well circumscribed mass of endometrial tissue was found in the wall of the appendix near its tip, separating the fibers of the muscularis and showing the characteristic decidual reaction of pregnancy.

The convalescence was uneventful. There was no recurrence of abdominal pain, the patient continued normally through the period of gestation and was delivered of a normal full-term child in April 1935.

ABERRANT PANCREATIC AND INSULAR TISSUE IN THE WALL OF THE JEJUNUM ROBERT W. MATHEWS

In a colored man, aged 33, who died in the Philadelphia General Hospital of pulmonary tuberculosis with spread by way of the intrapulmonary blood stream, an unidentified lesion was found in the terminal part of the jejunum. It was almond-sized, firm, grayish yellow and covered by intact mucosa and serosa. On microscopic examination this mass was found composed of aberrant pancreatic tissue, the acini being of normal size and appearance, and the ducts numerous and of irregular size and position, several islands of Langerhans were identified. The gland occupied the whole of the submucosa and muscularis, the mucosa and serosa were intact.

The pancreas is derived from the ectoderm of the primitive alimentary tube, beginning about the sixth week, and is formed from two anlagen opposite each other, the dorsal and the ventral pancreas. The dorsal pancreas is the larger and finally forms the body, tail and duct of Santorini. The ventral pancreas forms the head and the duct of Wirsung. The aberrant tissue was probably drawn away from the main mass in the alimentary tube and, with the lengthening of the tube, was carried to the anomalous position. Aberrant pancreatic tissue is said to be a common finding in the stomach and intestines, but in the Philadelphia General Hospital from 1920 to 1934 inclusive (23,543 necropsies) only two other such conditions have been recorded, one occurring in the stomach and the second in the lateral wall of the duodenum.

TUMOR IN THE WALL OF THE JEJUNUM COMPOSED OF PANCREATIC TISSUE P. R. TROMMER and J. C. SIMPSON

A white man, aged 32, was admitted to the Montgomery Hospital, Norristown, Pa., on several occasions over a period of fourteen years with severe symptoms of peptic ulcer. On his last admission in April 1934, operation by Dr. Donald M. Headings revealed the healed scar of a duodenal ulcer. A small tumor was found in the antimesenteric wall of the jejunum, 40 cm from the ligament of Treitz. It was 1.5 by 1 cm in diameter, light brown and of granular appearance and impinged slightly on the lumen of the bowel.

Posterior gastro-enterostomy and excision of the jejunal tumor were performed. The patient made an uneventful recovery. Since then he has been fairly well with the exception of two mild attacks of abdominal discomfort, these passed off quickly and caused no disability.

Microscopically, the tumor was composed of pancreatic tissue. Islands of Langerhans were not present. A definite duct lined with columnar epithelium was found in that part of the tumor situated beneath the mucous membrane of the jejunum.

INFLAMMATION IN SYPHILITIC AND NONSYPHILITIC RABBITS JOSEPH D. ARONSON

Rabbits infected intratesticularly with the Nichols strain of *Spirochaeta pallida* were given, two months later, intracutaneous injections of tubercle bacilli of the bovine type, staphylococci (*Staph. aureus*) or India ink. As controls, nonsyphilitic

rabbits were given similar injections. At varying intervals the sites of injection were removed for inspection.

Dr David Meranze and I noted that in syphilitic rabbits the injection of tubercle bacilli induced a perivascular polymorphonuclear infiltration, followed by sharply defined aggregations of mononuclear cells. The lesions resembled those observed in the skin of rabbits infected intracutaneously with *S. pallida*. Numerous fibroblasts appeared, throughout the experiment, with fewer epithelioid cells. In the nonsyphilitic rabbits the polymorphonuclear infiltration was diffuse, and was followed by the formation of an abscess and later by a sharply defined large tubercle. Numerous epithelioid cells and fewer fibroblasts were observed.

The injection of staphylococci into the skin of a syphilitic rabbit produced an extensive inflammatory reaction with marked edema, followed by an abscess. There was extensive infiltration by polymorphonuclear cells, which were arranged in aggregations in the tissue spaces, followed by a large abscess and slough, with but little granulation tissue. In the nonsyphilitic rabbits the inflammatory reaction was less marked. Sections of skin from these rabbits showed marked infiltration by polymorphonuclear cells and edema, followed by a small abscess and a small amount of granulation tissue seven days after inoculation.

In the syphilitic rabbits given injections of india ink, a well marked inflammatory reaction was observed, and on gross examination the india ink was found distributed irregularly throughout the derma. At first many aggregated polymorphonuclear cells were observed. Later carbon particles were found in mononuclear cells and in fibroblasts, and marked increase of connective tissue followed. On the other hand, in nonsyphilitic rabbits, no gross inflammatory reaction was seen, and on gross examination a sharply defined narrow brown line was noted. Microscopically, a slight cellular infiltration was observed, and the india ink, at first localized, was later found to be somewhat dispersed.

PATHOLOGY OF LYMPHOGRANULOMATOSIS INGUINALIS FRED W WEIDMAN

Anatomically there are two features for the pathologist to follow. 1 There is a rough parallelism with syphilis, i. e., a primary sore (often overlooked), followed by the bubo, with generalization of that disease (eye, spinal fluid) later. 2 The localization of the bubo is contingent on the differing distribution of the lymphatics of the external genitalia of the two sexes. Thus, in females, the extension is generally deep into the pelvis, inducing, among other things, rectal stricture.

As to etiology, the presence of virus appears to be thoroughly established.

Points of immediate practical significance to the pathologist of the general hospital are as follows. 1 Requests for diagnosis are likely to come from the gynecologist and the urologist, as well as from the general surgeon, the internist and the dermatologist. 2 The histologic features are sometimes distinctive, because the architecture of the lymph node is sometimes destroyed, on the other hand, the lesion may consist only of a diffuse round cell infiltration. When abscesses or tubercles are interspersed the picture is at least suggestive and may even be distinctive for lymphogranulomatosis inguinalis. 3 The Frei antigen, which is so specific, is not on the market, and every effort must be made to establish contact with the clinician for the purpose of securing the maximum amount of pus possible with which to make the antigen. Recent reports promise relief from this difficulty through employing brains of experimentally infected monkeys and mice and perhaps also cultures of the virus.

HISTOLOGY OF THE HEMOPOIETIC TISSUES IN HEMOPHILIA R P CUSTER and E B KRUMBHAR

Three fatal cases of hemophilia in which necropsies were made are reported. One patient died from uncomplicated hemorrhage, one from appendicitis and hemorrhage, and one from fulminating pneumonia. The hematopoietic tissues in all three cases showed normal regenerative ability, in the first two predominantly erythroblastic, in

the third leukoblastic. In all three cases there was a marked increase in megakaryocytes and their progenitors in the bone marrow, indicating a relationship of the blood platelets to the hemophilic process. J. H. Wright's observation of the formation of platelets in the sinusoids of the bone marrow from intruding pseudopods of the cytoplasm of megakaryocytes is supported by our observations.

This work is reported in full in the *American Journal of the Medical Sciences* (189 620, 1935)

NEW YORK PATHOLOGICAL SOCIETY

May 23, 1935

WILLIAM C. VON GIAHN, *Presiding*

CARCINOMA OF THE ULTIMOBRANCHIAL BODY RUBIN POLFSHUCK (by invitation)

A 28 year old man, an American, was admitted to the medical service of the Cumberland Hospital on July 31, 1934, with swelling of the neck, anorexia and vague gastro-intestinal disturbances.

The past history of the patient and family history were irrelevant.

Physical examination revealed a blond white man whose face and neck were full but not grossly edematous. The upper part of the chest presented numerous enlarged and tortuous veins. An x-ray film showed a nonpulsating shadow indicative of a tumor in the upper mediastinal area. Under the impression that the condition was Hodgkin's disease, the patient was given roentgen therapy and discharged to the clinic considerably improved, with, however, no decrease in the size of the tumor.

In the clinic he complained of occasional headaches with blurring of vision, stiffness of the neck and dizziness and weakness, which had caused him to fall down on several occasions. Physical examination revealed thrombosis in the external jugular vein on the right side and increased engorgement of all the veins of the chest, neck and arms.

The patient was readmitted to the hospital Jan 21, 1935, with the added complaint of dyspnea on exertion. Surgical removal of the mass was then attempted, and an inoperable tumor was found. Biopsy resulted in a diagnosis of carcinoma possibly bronchogenic in origin. The patient reacted poorly, pyopneumothorax developed, and death occurred on the tenth day after the operation.

The observations at autopsy were as follows. The anterior and superior mediastinum contained a large encapsulated nodular mass about 15 cm in diameter. This extended superiorly as far as the thyroid gland, posteriorly almost to the spine and anteriorly to the sternum, to which it was adherent. Inferiorly it extended to the heart, pushing the latter to the left, and laterally it encroached on both pleural spaces (more on the right one), where it was adherent to both lungs.

There was massive thrombosis in both the internal jugular and the subclavian veins, the thrombi extending into the tributaries of both of the arms.

The cut surface of the tumor was variegated, some areas being yellow and others gray or deep red. Some portions were soft, others were firm, and still others almost cartilaginous.

Microscopically, the tumor was made up of two rather definite types of cells. One was an elongated columnar cell, staining dark, which tended in many areas to form acini. The cytoplasm stained deep pink and was smooth. The nucleus varied in shape and size but was usually oval and vesicular. The other type of cell bore a striking resemblance to a plasma cell but was larger. Its cytoplasm was pink and smooth, and its nucleus, eccentric, hyperchromatic and round or oval. Numerous mitotic figures and dividing forms were seen. These cells were arranged in histoid fashion and were separated by a scant fibrovascular stroma.

The tumor as a whole was very vascular, and in some areas it appeared angiomatic, with numerous areas of hemorrhagic extravasation. The walls of the blood vessels were thin. Many vessels contained platelet thrombi, some of which showed hyalinization and calcification. A characteristic feature of the growth was its tendency to invade the lymphatics and blood vessels. In some sections the neoplasm also showed a tendency to grow in strands or groups of deeply staining cells separated by myxomatous and hyaline substance in which were many young fibroblasts. Large portions of the tumor showed degenerative changes and calcium deposition. Other portions showed areas of necrosis with peripheral infiltration by polymorphonuclear cells.

The pericardial sac contained about 1,500 cc of a cloudy straw-colored fluid. The epicardium was covered by fibrinopurulent exudate. The neoplasm extended into the upper portion of the pericardium.

The right pleural cavity was filled with a large amount of fibrinopurulent exudate compressing the lung.

The posterior mediastinal and supraclavicular lymph nodes were enlarged and on microscopic examination showed metastatic groups and strands of cells similar to those of the main tumor mass.

Although the history of this case as well as the location of the tumor suggests the possibility of a thymic neoplasm, still the histologic structures fail to fulfil the criteria for this type of growth and suggest an origin from an epithelial structure such as a branchial rest. References to this type of neoplasm in the literature are scant. Getzowa, in a study of serial sections of thyroids from idiots and cretins, found many tubular structures and solid groups of cells which histologically belonged neither to the thyroid nor to the parathyroid. These she considered to be rests of the ultimobranchial bodies.

Langhans described five cases of tumor of the ultimobranchial body. In each of these cases the structure of the tumors, he believed, resembled closely the cell groups described by Getzowa.

Ewing also described two cases of a similar tumor which he believed to have originated in the ultimobranchial body, and in our case examination of the gross and microscopic specimens suggested the possibility of a similar origin.

DIFFERENT FORMS OF LEIOMYOSARCOMA UTERI LOUISE H. MEEKER

Myoma of the uterus furnishes 50 per cent of all tumors. Less than 2 per cent are malignant. Four specimens differing in gross morphology are presented: (1) a large pedunculated form, (2) a circumscribed lymphangiectatic form, (3) a circumscribed cystic type and (4) a diffuse form. They do not differ in minute histology, and the gross difference seems to depend largely on the situation of the primary focus and its relation to the blood vessels and lymphatics in the associated connective tissue. Types 3 and 4 deserve special mention.

In type 3, the cysts are usually produced by dissolution of connective tissue and accumulation of lymph, which pushes the muscle nodes to the periphery of the tumor. Cavities of the type I am presenting are exceedingly rare. Cullen stated in 1909 that there was only one tumor of this type among 1,674 examples of myoma of the uterus reviewed by him. Helher in 1914 published a report of such a tumor which he considered remarkable. The inner wall was marked by prominent gyri and was lined by many layers of closely packed smooth muscle cells. Other examples are wanting or are not described in histologic detail. All the cystic forms of this type are described as of the size of a grapefruit.

In type 4, the connective tissue of the myometrium in general shows lymphangiectasis, and the multiple foci of the myoma form ramifying tumor pegs throughout the distended lymphatics (a modification of myoma gyratum?). Few tumors of this type have been reported. There are descriptions of such tumors, but the details are more or less vague. Examples are described in reports of R. Meyer, and several are cited by him. Boyd has said that the type is so rare that it need not be considered. Frank has described similar ramifying growths under the title

"Fibromyomasis—an Unclassified Plexiform Endolymphatic Proliferation of the Uterus" In the one which I show there is no glandular epithelium or mucosal stroma to suggest origin in a previous adenomyoma

Although these four examples of leiomyosarcoma of the uterus differ in gross structure they are the same in cell type. The neoplastic cell in each instance is a small round or spindle-shaped cell with rounded nucleus and scanty cytoplasm. It does not exhibit polymorphism, and mitotic figures are not abundant. The active cell is a muscle cell of embryonal type, and the connective tissue does not participate in the neoplastic process.

The greatest variation is seen in the degeneration and liquefaction of the connective tissue and the relation of this to the lymph stasis. I find abundant hyaline degeneration and extensive liquefaction in both the tumors and the connective tissue of the general myometrium of the diffuse growths. The myoblasts are most abundant where the lymph is most abundant both in the partly liquefied matrix and in the lymph channels.

One is reminded that the connective tissue of the uterus contains embryonal smooth muscle cells that multiply greatly during pregnancy. That these cell rests may give rise to tumors has been suggested and seems probable. If connective tissue secretes a hormone, as claimed by several investigators, doubtless the extremely labile connective tissue of the uterus has its own type of hormone to balance the hormonal stimulus which promotes activity of uterine muscle, and a change in the drainage and the amount of lymph may upset this balance, allowing one or the other tissue to predominate. Such a condition may be local or under certain circumstances widespread, and multiple tumors arising from the muscle rests may produce the diffuse sarcoma of muscle origin.

DISCUSSION

ALFRED PLAUT In the last ten years, my associates and I have seen only two examples of leiomyosarcoma of the uterus. I agree with Dr Meeker that the percentage given in the older literature is entirely too high. One can easily make a test by carefully choosing a high power microscope field of an ordinary cellular myoma uteri and asking a younger pathologist what he calls that. Quite often one will hear the diagnosis sarcoma.

I should like to ask Dr Meeker whether her last case is supposed to be similar to the case which Dr Robert T Frank presented several years ago at the Graduate Fortnight of the New York Academy of Medicine. If I remember correctly, the cells of that tumor, of a kind which I had never seen before, or after, seemed to conform to those of the stroma of the endometrium more than to muscle cells. If I understood Dr Meeker correctly, I heard her refer to Dr Frank's case, and I wonder what the relation of these two types of tumor may be. I also should like to ask how far the gross appearance of Dr Meeker's tumors was suggestive of sarcoma. Did the cut surfaces have the characteristic look of cut crab meat or fish meat?

S H POLAYES Was the endometrium involved in all of these four cases, or just in one?

NATHAN C FOOT Can Dr Meeker give any statistics of the likelihood of metastases? As I understand it, it is extremely rare for sarcoma of this type to metastasize, but one is often confronted by the surgeon who asks about the likelihood of metastases in such cases.

LOUISE H MEEKER In reply to Dr Plaut, I should like to say that I think Dr Frank intended to convey the idea that the stromal cells of the endometrium were the active cells in his tumor. The cells in such a case are, according to the opinion of Ewing, embryonal muscle cells.

As to the tumors having the characteristic appearance of sarcoma, that is questionable. They looked as though they might be sarcoma, but I do not know that I could say they did positively, except in the case of the diffuse tumor, which certainly suggested sarcoma.

In regard to involvement of the endometrium, there was no involvement of it except from pressure, which produced the polypoid thickenings. The idea that endometrium may have extended into blood vessels, as Sampson thought, to give origin to the tumor of diffuse type and that then the glandular elements were destroyed by pressure does not seem to be borne out here. There was nothing to suggest any relation to the endometrium or to the endometrial stroma. It was all embryonal muscle which produced the active proliferating tumor.

In answer to Dr. Foot, tumors of this type sometimes metastasize, but metastasis is very rare. The surgeons who operated in these cases are questioning what will happen before the end of five years.

LEIOMYOSARCOMA OF THE GALLBLADDER LOUISE H. MEEKER

The specimen is presented because of the rarity of muscle sarcoma of the gallbladder. It is from a case included by Dr. John F. Erdmann in his recently published article, "Neoplasms of the Gall Bladder." Few cases of undoubted sarcoma of the gallbladder have been reported. In only a small number of these was the tumor derived from muscle. Gaetani's summary of thirty reputed cases, published in 1932, included only one of leiomyosarcoma. The microscopic picture is that of closely packed large embryonal muscle cells, varying in shape from round to polyhedral, with fairly frequent mitotic figures.

FILTRATION AND SECRETION IN THE HUMAN KIDNEY HOMER SMITH (by invitation)

It is supposed that the remote ancestor of the vertebrates possessed a tubular kidney draining the body celom, such as is observed in most invertebrates and especially in the annelids. The glomerulus, which is found only in the kidney of the vertebrate, is supposed to have been evolved by some primitive chordate to facilitate the excretion of water from the body. This postulate rests in part on the currently accepted views of renal function and in part on the paleontological evidence that the earliest definitive fishes were inhabitants of fresh water. The first glomerular forms, it should be noted, may well have been in highly organized vertebrates; the postulate does not require, as Chamberlain has suggested, that the first chordates themselves were evolved in fresh water, but only that the special character of the glomerular kidney indicates a fresh water habitat at the time when this character was established.

So long as the organism remained in fresh water (dipnoans, ganoids and teleosts) or in intimate dependence on it (amphibians) this excretory arrangement persisted, but with the secondary assumption of a marine habitat (teleosts) where the osmotic gradient was reversed and the excretion of water reduced, or with the assumption of terrestrial life, in which conservation of water became a necessity (arid-living reptiles and birds), the organism no longer needed and could no longer economically use this primitive water-excreting mechanism. There was thus a need to either (a) discard or reduce the glomeruli or (b) amend their primitive function by adding distally a more efficient mechanism for the reabsorption of water. The first process appears to be occurring in the marine teleosts and in the reptiles. In the mammals and possibly to some extent in the birds, on the other hand, the addition of the loop of Henle has permitted the reabsorption of water against the osmotic pressure of the metabolites in the urine, consequently in these the glomeruli, although still very active as filters, have become secondarily incorporated into a filtration-reabsorption system which permits the excretion of waste products without the excretion of excessive quantities of water.

In man the quantity of glomerular filtrate (glomerular clearance) is about 100 to 125 cc per minute. The urea clearance is considerably lower, probably because of reabsorption some place along the tubular system. The creatinine clearance is higher by about 40 per cent, and the phenol red clearance is more than three times as great, in both instances because of tubular excretion from the postglomerular blood. Other substances which are normally absent from the urine (dextrose, creatine, etc.) show an increasing clearance as the level of the plasma is raised.

DISCUSSION

ALFRED PLAUT Is there a special behavior of the glomerular apparatus of fish which change from sea water to fresh water and vice versa, for instance, the eel, which leaves the depth of the mid-Atlantic and migrates to the estuaries of America and Europe, with the young returning from the rivers to the original place?

HOMER SMITH Dr Plaut's question touches subjects which are being investigated. It seems, *a priori*, that there must be a physiologic mechanism for reducing glomerular activity when fishes go from fresh into salt water. There are observations which indicate that the glomeruli of the eel, in particular, are under physiologic control, but how that control operates, I do not know. It has been suggested for frogs and fish that there are shunts between the afferent and efferent arterioles so that the blood can be sent through without crossing the entire capillary bed. That might be one method. Another method might be that of an ischemia of the kidney. Nothing is known about the details of the mechanism involved, and my associates and I are interested in finding out whether such physiologic mechanisms condition the migration of fish like the eel from fresh water into salt water.

THE THIRD DIMENSION IN PATHOLOGIC INVESTIGATION JEAN OLIVER (by invitation)

The importance of the third dimension in investigations of morphologic changes in diseased tissues was illustrated by the demonstration of plastic preparations of the abnormal nephrons of the kidney in chronic Bright's disease. These structures were shown in model reconstructions by the Born wax plate method and in microdissections of tissue macerated in strong hydrochloric acid. The various changes that have been observed in the course of the nephron from the glomerulus to the pelvis of the kidney were shown (ARCH PATH 15 755, 1933, 18 777, 1934, 19 1, 1935) and attention drawn to the possible use of these methods in the investigation of other morphologic problems.

DISCUSSION

HOMER SMITH I should like to ask what proportion of glomeruli might still be functioning in the kidney, in a case of extremely severe chronic glomerulonephritis, or what proportion of aglomerular tubules, when the glomeruli are gone. Is it 1 in 100,000, 1 in 1,000 or 1 in 10?

JAMES A. SHANNON What proportion of the glomeruli in pathologic cases have hypertrophy of Ludwig's vessel? Is it a common thing?

JEAN OLIVER In regard to the frequency of occurrence of aglomerular tubules I should reply that my associates and I are still working on this problem, and I do not think I am prepared as yet to make a definite statement about it. The answer is complicated by the fact that chronic glomerulonephritis seems to produce two architectural forms of kidney. In one there is a very diffuse change characterized by simple atrophy of the proximal convolutions, and in that form it is only very occasionally that one finds aglomerular tubules. This sort of kidney is usually found when the disease has been fairly rapid in its development, or perhaps it would be better to say, when it shows high intensity throughout its course—when there are no remissions. The other type of kidney is quite different, because the changes are decidedly patchy. There are areas that are similar to what I have described, that is, where atrophy is the outstanding change in the nephrons, but there are other areas in which the nephrons are not only well preserved, but are hypertrophic and hyperplastic. You have all seen these two pictures in sections of kidney showing chronic glomerulonephritis. One is the smooth kidney of Fahr, and the other is the nodular kidney. It is in the latter type, the type in which the disease has gone on for years—the patient has shown remissions, he has been quite ill for long periods of time and then apparently well for weeks or months—

that one finds aglomerular tubules. But I do not think I can give any accurate figure as to what the proportion is, though I can say they are common. One can find them without any difficulty if one looks in one of the better preserved portions of the kidney.

I have the same difficulty in answering the second question as to how frequent the occurrence of Ludwig's vessel is. It is practically impossible to find this vessel in normal kidneys. In fact, in a normal kidney I have never found one that I was certain of. Moreover, there is a practical difficulty in identifying the vessel of Ludwig and this comes from the fact that the afferent vessel may subdivide and go to two or more glomeruli, if one breaks off a branch and does not know one has broken it off, one has something that apparently fulfils the requirements of Ludwig's vessel. The real vessel of Ludwig comes off at almost a right angle from the afferent vessel. If one has been careful, one knows there never was a glomerulus there. If one is fortunate, there are capillaries on the end of the vessel. In that case there is no question but that the finding is Ludwig's vessel. But in the normal kidney it is almost impossible to find an example. In abnormal kidneys it is fairly easy to find examples, but again I hesitate to give a figure. It would mean dissecting several hundred specimens, and one would have to be sure in the several hundred that one had prepared that one had not missed any vessel of Ludwig. Then, of course, if one were sure of one's facts, one could give a statistical figure, but this would be a difficult thing with this technic. I think it is permissible to say that Ludwig's vessel and the aglomerular tubule are found frequently, but I do not think I dare say much more than that.

HOMER SMITH. In the illustration shown do you know whether that instance of Ludwig's vessel goes to the proximal or to the distal tubule?

JEAN OLIVER. It goes to the neighborhood of both. I do not think I can say it goes to one, because the two tubules are so mixed.

Book Reviews

La protidémie et la pression osmotique des protides Recherches expérimentales et applications cliniques By Antoine Codounis, Professeur agrégé à la Faculté de Médecine d'Athènes Preface by Professeur Ch Achard Paper Price, 36 francs Pp 212 Paris Masson & Cie, 1934

Prior to his appointment at the University of Athens, Codounis was associated with Achard in Paris. He published with Achard and with other collaborators a long series of articles on various aspects of the same subject with which this book deals. This work, which received the prize of the Academy of Medicine in Paris, is a summary of all those publications, with brief references to the work of other authors. Introductions by Achard and Codounis occupy six pages.

The first part, of fifty-five pages, deals with experimental investigations. It begins with a presentation of methods, particularly of the author's own technique of the determination of protein. Then follow discussions: (a) of the properties of serum protein and of its fractions, (b) of the origin of these fractions and of their relation to the liver, lungs, spleen, kidneys, stomach and thyroid, (c) of the influence of cathartics and of thirst on the blood proteins of the dog. Very valuable is the next chapter on the blood proteins in normal animals that are commonly employed for experimental work (the horse, the rabbit, a few species of monkeys, the dog and the guinea-pig). Results of studies dealing with the influence on blood proteins of experimental amyloidosis and experimental intestinal obstruction in the dog, of immunization and of various experimental infections in the horse and of infections in the monkeys are recorded.

The major part of the book, consisting of one hundred and fifteen pages, is devoted to a presentation and analysis of a large mass of data dealing with the variation of blood proteins in various diseases of man. The series includes twenty-two normal healthy persons, twenty pregnant women, ten new-born babies and three hundred and thirty patients afflicted with various diseases. The examinations included an estimation of osmotic pressure, total proteins, albumin, globulin, the albumin-globulin ratio and, in some cases, lipoids and cholesterol. The finding of a reversal of the normal albumin-globulin ratio, owing to a lowering of the total proteins, but particularly of the albumin, in various acute and chronic infectious diseases, confirms a few older reports. In striking contrasts are the normal or slightly elevated values for protein in tuberculous meningitis, while a similar change is present in pulmonary tuberculosis and in other infectious diseases. The prognostic value of the low protein level is illustrated with histories of cases. A study of fifty-one cases of various forms of nephritis and of nephrosis, with the clinical and laboratory data, should prove of value to clinicians and investigators, even if they would not agree with the conclusions of Codounis.

The chapters dealing with the diseases of the heart and blood vessels and of the blood, the liver, the digestive tract, the endocrine glands and the nervous system and with malignant tumors contain valuable observations. Codounis concludes this section with the significant remark that the disturbances of the protein balance are a nonspecific phenomenon similar to the sedimentation reaction of erythrocytes. That impression forces itself all along on the reader. If the different and laborious chemical estimations add little or nothing to the information offered by the simple sedimentation test of erythrocytes, the choice between the two should be easy.

The book closes with a short chapter on the osmotic pressure of proteins and its significance in pathology. The bibliography lists one hundred and eighty-two titles and is obviously incomplete with regard to German and American publications, among which even fundamental recent articles were omitted. On the other hand, the publications of Achard and of his associates take up about one third of the whole list. The American student of the subject will find the accumulated laboratory data of considerable value, but if he seeks information, according to the

reviewer, he will find more of it, more clearly presented and more easily accessible, in the sixty pages of chapter XIII in the first volume of Peters and Van Slyke's "Quantitative Clinical Chemistry"

The Pathology of Internal Diseases By William Boyd, M D, M R C P (Edin), F R C P (Lond), Dipl Psych, F R S (Can), Professor of Pathology in the University of Manitoba, Pathologist to the Winnipeg General Hospital. Second edition, thoroughly revised. Price, \$10. Pp 904, with 335 illustrations. Philadelphia. Lea & Febiger, 1935

It is a pleasure to welcome the second edition of this book. Major alterations have been made in many sections, and much new material has been added. The references at the end of the chapters are now arranged under subjects in place of alphabetically according to the authors' names, which is of advantage when the titles to articles in periodicals are omitted. "The aim of the present edition has been to develop the subject along clinical and physiological lines even more than to add material which is pathological in the strict sense of morbid anatomy." The scope of the book is best shown by the subjects of its seventeen chapters: diseases of the heart, arteries, respiratory system, stomach and duodenum, intestines, liver and gallbladder, pancreas, kidneys, adrenal glands, thyroid gland, parathyroid glands, pituitary body, blood, bone marrow, spleen, lymph nodes and thymus gland and nervous system. The book consequently covers the field usually included under the term internal medicine, except that acute infectious diseases such as measles, scarlet fever, smallpox, tularemia, undulant fever and whooping cough have not been included. This omission will be a disappointment to many readers. As indicated by the quotation from the preface, the general plan is to discuss the nature, the etiology and the structural changes of internal diseases, with special reference to the relations between the clinical manifestations and the lesions. The illustrations answer their purpose well. The style is clear and fluent. There may be a slight tendency to resort to general phrases to explain obscure phenomena. This is illustrated by the following statement from the section on sudden cardiac death: "Finally there remains a group of cases in which the patient dies of sudden heart failure, but no satisfactory cause can be assigned. Such cases may be put down to shock, status lymphaticus or a visitation from God." And in discussing infectious processes probably too many effects are ascribed offhand to "the action of the toxin." The statement on page 296 that food-handlers constituted the chief danger in the Chicago outbreak of amebic dysentery in 1933 should not be allowed to stand, because the main factor in that outbreak was contamination of the water supply in two hotels. Under heart block (p 63) it is stated that the course of the conduction system is described at the beginning of the chapter, but it appears that in the revision of the first edition that description has been omitted.

The book is an excellent introduction to the study of clinical medicine and will be of special interest to medical students as they enter clinical work as ward clerks and interns, as well as to physicians who wish to refresh their knowledge of the pathology in the broad sense of internal diseases.

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A BIBLIOGRAPHY OF TWO OXFORD PHYSIOLOGISTS RICHARD LOWER, 1631-1691, JOHN MAYOW, 1643-1679 John F Fulton, M A Oxon, Sterling Professor of Physiology in the Yale University School of Medicine Pp 62, with 7 figures London Oxford University Press, 1935

THE DETERMINATION OF IODINE IN BIOLOGICAL SUBSTANCES Medical Research Council, Special Report Series, No 201 C O Harvey Price, 1 shilling Pp 43 London His Majesty's Stationery Office, 1935

FORENSIC MEDICINE A TEXT-BOOK FOR STUDENTS AND A GUIDE FOR THE PRACTITIONER Douglas J A Kerr, M D, F R C P E, D P H, Lecturer on Forensic Medicine in the School of Medicine of the Royal College, Edinburgh, The Police Surgeon and Medical Referee to the City of Edinburgh Price, \$5 50 Pp 311, with 40 plates, 4 color plates and 30 other figures London A & C Black, Ltd (New York The Macmillan Company), 1935

A TEXTBOOK OF LABORATORY DIAGNOSIS WITH CLINICAL APPLICATIONS FOR PRACTITIONERS AND STUDENTS Edwin E Osgood, M A, M D, Assistant Professor of Medicine and Biochemistry, Director of Laboratories, University of Oregon Medical School Second edition Price, \$6 Pp 585, with 27 text figures and 10 colored plates Philadelphia P Blakiston's Son & Co, 1935

AGENTS OF DISEASE AND HOST RESISTANCE, INCLUDING THE PRINCIPLES OF IMMUNOLOGY, BACTERIOLOGY, MYCOLOGY, PROTOZOOLOGY, PARASITOLOGY AND VIRUS DISEASES Frederick P Gay and Associates Price, \$10, Pp 1581, with 212 figures, 6 color plates and 60 diagrams Springfield, Ill Charles C Thomas, publisher, 1935

HUMAN PATHOLOGY A TEXTBOOK Howard T Karsner, M D, Professor of Pathology, Western Reserve University With an introduction by Simon Flexner, M D Cloth Fourth edition, revised Price, \$10 Pp 1013, with 18 illustrations in color and 433 in black and white Philadelphia J B Lippincott Company, 1935

A TEXTBOOK OF GENERAL BACTERIOLOGY Edwin O Jordan, Ph D, Professor of Bacteriology in the University of Chicago and in Rush Medical College Eleventh edition Cloth Price, \$6 Pp 825, with 202 illustrations Philadelphia W B Saunders Company, 1935

CLINICAL PARASITOLOGY AND TROPICAL MEDICINE Damaso de Rivas, B Sc, Biol, M S, M D, Ph D, Professor of Parasitology in the Graduate School of Medicine and Assistant Professor in the Department of Pathology, University of Pennsylvania In collaboration with Carlos T de Rivas, B A, M D, Pathologist to the Santo Tomas Hospital, Panama Price, \$5 Pp 367, with 145 illustrations Philadelphia Lea & Febiger, 1935

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